

**NEUROPHARMACOLOGICAL EFFECTS OF METHANOLIC EXTRACT OF
LEAVES OF *AMARANTHUS VIRIDIS*.L IN RATS & MICE”**

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MASTER OF PHARMACY
IN
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Submitted by
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OCTOBER - 2017

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitle “**NEUROPHARMACOLOGICAL EFFECTS OF METHANOLIC EXTRACT OF LEAVES OF *AMARANTHUS VIRIDIS*. L IN RATS & MICE**” submitted by **Register No: 261525504** to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfilment for the degree of **MASTER OF PHARMACY in PHARMACOLOGY** is the bonafide work carried out under guidance and direct supervision of **Mr. P.ROYAL FRANK, M. Pharm., Assistant Professor** at the Department of Pharmacology, **THE ERODE COLLEGE OF PHARMACY AND RESEARCH INSTITUTE, ERODE-638112** and was evaluated by us during the academic year 2016-2017.

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DECLARATION

I do hereby declare that the dissertation work entitled “**NEUROPHARMACOLOGICAL ACTIVITY OF METHANOLIC EXTRACT OF *AMARANTHUS VIRIDIS*. L PLANT IN RATS**” submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in the partial fulfilment for the Degree of **MASTER OF PHARMACY in PHARMACOLOGY**, was carried out by myself under the guidance and direct supervision of **Mr. P.ROYAL FRANK, M. Pharm., Assistant Professor**, at the Department of Pharmacology, **THE ERODE COLLEGE OF PHARMACY AND RESEARCH INSTITUTE, ERODE-638112**, during the academic year 2016-2017.

This work is original and has not been submitted in part or full for the award of any other Degree or Diploma of this or any other University.

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CONTENTS

SL.NO	CONTENTS	PAGE. NO
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	53
3.	PLANT PROFILE	63
5.	AIM AND OBJECTIVES	68
6.	PLAN OF WORK	69
7.	MATERIALS AND METHODS	70
8.	RESULTS AND DISCUSSION	89
9.	SUMMARY AND CONCLUSION	113
10.	FUTURE AND PROSPECTIVES	114
11.	BIBILIOGRAPHY	115

LIST OF TABLES

SL.NO	CONTENTS	PAGE.NO
1.	Neurotransmitters found in nervous system	8
2.	General description of established antiepileptic drugs	24
3.	General description of New anticonvulsants	26
4.	Data showing colour, consistency and yields of methanolic extract of powdered leaves of <i>A. viridis</i> Linn	89
5.	Data for ash values for powdered leaves of <i>A. viridis</i> .L	96
6.	Data for extractive value and loss on drying of powdered leaf of <i>A. viridis</i> L	96
7.	Results of the phytochemical constituents of methanolic extract of <i>A. viridis</i> .L	97
8.	Effect of methanolic extracts of <i>A. viridis</i> .L. on maximal electroshock induced in rats	98
9.	Effect of methanolic extracts of <i>A. viridis</i> .L on forced swimming test in mice	101
10.	Effect of methanolic extracts of <i>A. viridis</i> .L on Tail suspension test in mice	103
11.	Effect of methanolic extract of <i>A. viridis</i> .L on elevated plus maze test in mice	105
12.	Effect of methanolic extract of <i>A. viridis</i> .L on light - dark model in mice	107

LIST OF FIGURES

S.NO	CONTENTS	PAGE.NO
1.	Neuronal sites of action of antiepileptic	14
2.	Sites of action of antiepileptic in GABAergic synapse	15
3.	Mechanism of action of Phenytoin	31
4.	Monoamine hypothesis of depression	36
5.	General mechanism of action of Anti depressant drug	40
6	Mechanism of action of TCAs	43
7.	Schematic depiction of GABA _A benzodiazepine receptor-chloride channel complex	48
8.	Plan of <i>A. viridis</i> .L	63
10.	T.S of petiole of leaves of <i>A. viridis</i> .L	90
11.	T.S of midrib of leaves of <i>A. viridis</i> .L	91
12.	T.S of the lamina of the leaf of <i>A. viridis</i> .L	93
13.	Venation pattern of leaves of <i>A. viridis</i> .L	94

14.	Diagrammatic representation of effects of methanolic extract of <i>A.virdis</i> on maximal electroshock induce in rats	99
15.	Diagrammatic representation of effects of methanolic extracts of <i>A. viridis.L</i> on forced swimming test in mice	102
16.	Diagrammatic representation of effects of methanolic extracts of <i>A. viridis.L</i> on tail suspension test in mice	104
17.	Diagrammatic representation of effects of methanolic extracts of <i>A. viridis.L</i> on elevated plus maze test in mice	106
18.	Diagrammatic representation of effects of methanolic extracts of <i>A. viridis.L</i> on light – dark model in mice	108

1. INTRODUCTION

Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants. Medicines derived from plants are widely famous due to their safety, easy availability and low cost. Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seed and flowers of plants. They are administered orally, inhaled or directly applied in the skin. Medicinal herbs are more significant to the health of individual and community. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more. These natural compounds formed the foundations of modern prescription drugs as we know today. Use of plants based drugs for curing various ailments is as old as human civilization and is used in all cultures throughout history. The primitive man started to distinguish between useful and harmful or poisonous plants by trial and errors. A well defined herbal pharmacopoeia was developed by tribal people who were based on information collected from local flora, religion and culture. The knowledge of medicinal plants was gradually developed and passed on from one individual to other, which foundation for traditional medicine throughout the world.

Classification of herbal medicines:

Herbal medicines are classified into;

1. Ayurvedic Herbalism

(Ayurveda meaning science of life) which is derived from Sanskrit word. This system originated from Indian medicines about 500 years ago and was practiced in its neighboring countries like Srilanka.

2. Chinese Herbalism

3. African Herbalism

4. Western Herbalism, Greece and Rome were their originating countries which were then spread to Europe, North and South America. Plants based medicine play an important role in world health. Medicinal plants are distributed worldwide but they are very rich in tropical countries. Modern medicine from high plants either directly or indirectly derived is estimated 25%. Herbal medicines might be considered as “diluted drugs”. An adequate amount is required by an individual for specified period of time in order to achieve the desired benefits of herbal medicines. Every herb is different from other herb; some herbs are safe and effective for specified use while others are not. This is not a true preparation that the herbal medicines are very safe and free from any side effect because they can produce side effects and can be toxic. As each part of plant will have many active constituents and some of its constituents may be toxic, however, a high dose is required to cause toxicity because they are not potent. Also their adverse effects are relatively infrequent as compared to manufactured drug.

Until the nineteenth century, the main source of products used to maintain health were medicinal plants when accidentally Friedrich Wohler in 1928 synthesized urea while attempting to prepare ammonium cyanate from silver cyanide and ammonium chloride and this was the first organic synthesis in history and hence led to the era of synthetic compounds. With this discovery, the western scientists gave their full concentration to the newly discovered synthetic lines and ignored a little bit to the phytomedicines but after some time they were compelled to bounce back to the phytomedicines as they observed that the earlier has less side effects as compared to synthetic medicines. However, plants derived medicines are far superior to the well defined drugs. For example, the quality and availability of raw materials is always a problem, the principles of handling are also unknown and also the quality control, i.e. standardization and stability are practically applicable but not too much

easy. Herbal medicines are for superior than the synthetic drugs because they are naturally occurring, easily available without cost and have minimum side effects. Majority of plants have medicinal properties, i.e. most pharmaceutical drugs are originally derived from plants. The scientific study of indigenous medicines is called Ethno pharmacology, which is an interdisciplinary science practiced all over the world. Standardization herbal preparation is termed as phototherapeutic agents or phyto-medicine which contains active constituents, or complex mixture of plant materials in the raw or processed form. Phototherapeutic agents are usually not recommended to use in emergency treatment because of the fact that they normally do not possess an immediate or strong pharmacological action. The modern field of phytoscience comprise of the use of medicinal plants and their bioactive phyto-compounds. This science is developed from merging of vast range of disciplines that have never been linked before combining several different areas of economic, biochemistry, physiology, microbiology medicines and agriculture. The development and introduction of new drugs like antibiotics, immuno-stimulants and anti tumor agents have led to dramatic success in control of many diseases. The drugs derived from plants, however, still from the mainstay of medical treatment in the developing countries. According to the June 1983, issue of world Health, it is estimated that more than half of the world's population relies mainly on traditional remedies. Natural products and the medicinal agents derived there from, are also an essential feature in the health care system of the remaining of the population residing mainly in developed countries, More than 50% of all drugs in clinical use have a natural product origin. Natural products continue to play an important role in drug discovery programs of the pharmaceutical industry and other research organizations' More than 600 botanical items have been recognized in various editions of the United Sates Pharmacopoeia .The fact that most of the plant materials have been used over the ages for treatment of diseases is convincing evidence

that many of the herbal prescriptions are reasonable safe but scientific toxicological trials are still necessary ^[1].

MEDICINAL PLANT:

Importance and scope

Herbs are staging a comeback and herbal 'renasissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herb had been prized for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetic is over and people are returning to the naturals with hope of safety and security.

It has been estimated that in developed countries such as United States, plant drugs constituents as much as 25% the total drugs. While in fasting developing countries such as china and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to developing countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine. Of the 2, 50, 000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climate zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. This proportion of medicinal plants is the highest proportion of plants known for their medicals purpose in any country of world for the existing flora of that respective country. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, flower, seed , etc. Some drugs

are prepared from excretory plant product such as gum, resins and latex. Even the allopathic system of medicine has adopted a number of plants – derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (eg. Diosgenin, solasodine, beta-ionone). Not only that plant-derived drug offers stable market worldwide, but also plant continue to be important source for new drugs.

The Indian system of medicine is in the list approved by the National council for Alternative Medicine (NCAM), USA, although most of these drugs that claim to cure various ailments are yet to be validated scientifically. According to parasnis (2004), the full potentials of Ayurveda can be realized only by subjecting the ayurvedic drugs to modern investigation techniques. Further acceptance of any clinical trial depends on whether it satisfies modern pharmacological and statistical standards or not. Unfortunately, most people practising Ayurveda decline to adopt modern research techniques for evaluation of formulations used in the practices. This holds good for other traditional medical practitioners like those of siddha and unani medicinal systems too. Scientific validation will not only popularize these medicines in India but also render them acceptable, in some form to people in other parts of the world. Considering the fact that several disease do not have an ultimate answer in the conventional system whether in native regions or throughout the world, an effort to recognize the potential of alternative and combinational treatment systems validated through universally acceptable methods could provide be very beneficial for the human community at large^[2].

NEUROPHARMACOLOGY

Introduction:

Neuropharmacology is the study of how drugs affect cellular function in the nervous system, and the neural mechanisms through which they influence behaviour. There are two main branches of neuropharmacology: Behavioral and Molecular. Behavioral

neuropharmacology focuses on the study of how drugs affect human behavior (neuropsychopharmacology), including the study of how drug dependence and addiction affect the human brain.^[3]

Neuropsychopharmacology may be defined as the branch of inter disciplinary neuroscience devoted to the study of drugs that have an effect on the nervous tissue and alter the behaviour. Neuropharmacology deals with the study of effects of the drug on nerve cells, their synapses and circuit whereas the study of effects of drugs on behaviours, including emotional and cognitive mental activities, is Psychopharmacology. It associates the frontiers of fundamental neuroscience to the management of psychiatric and neurological diseases. This branch of science seeks to comprehend how drugs selectively affect the CNS to induce sleep, relieve pain, reduce fever, suppress muddled movement, prevent seizures or enhance attention. Neuropsychopharmacology seeks to understand how drugs can treat mania, anxiety, schizophrenia or depression without disturbing the consciousness. This field seeks to uncover the biological basis for intricate mental states. The goal of this field is not only to understand the nature of the alterations in biology which direct to distorted emotions and thought processes, but also to develop therapeutically priceless specific molecules which regulate the specific biologic underpinnings- namely, the as yet vague sequences of multi-neuronal interactions by which the behaviors emerge^[4].

Advance in modern science and technology have contributed to an enormous development in the quality of human life. However, modern life stresses are responsible for the surge in incidence of variety of psychiatric disorder. Drugs that are currently used in managing different neuropsychiatric and neurological disorders like anxiety, depression, schizophrenia, epilepsy, Parkinsonism either have severe side effects or posses inauspicious drug-drug/drug-food interactions. Moreover, the western system of medicine pays no heed to the fundamental problems and depends on drug treatment to cure the symptoms. The western

restorative treatment fails to tackle the huge diversity among patients. In this context, Ayurvedic treatment, which considers a patient's entire body-mind-spirit relationship and bio individuality which aims to look at the root cause of the disease and its relation with the lifestyle, thoughts and beliefs of the person (vital energy of the patient), have attained widespread fame in treating the neurologic disorders than the western system of medicine^[5]. Molecular neuropharmacology involves the study of neurons and their neurochemical interactions, with the overall goal of developing drugs that have beneficial effects on neurological function. Both of these fields are closely connected, since both are concerned with the interactions of neurotransmitters, neuropeptides, neurohormones, neuromodulators, enzymes, second messengers, co-transporters, ion channels, and receptor proteins in the central and peripheral nervous systems.^[3]

Neurotransmission

Neurotransmission may be defined as the process of transfer of impulses between neurons and Neurotransmitters are those biochemical substances responsible for the transmission of nerve signals across a synapse between two neurons.

The Central Nervous System uses a wide variety of Neurotransmitters including both Excitatory and Inhibitory chemical transmitters including:

1. Biogenic Amines

- Dopamine
- Norepinephrine
- Epinephrine
- Serotonine
- Histamine

2. Amino Acids

- Glutamic Acid
- Aspartic Acid
- Gama Amino Butyric Acid
- Glycine

3. Peptides

- Angiotensin
- Endorphins
- Vasopressin
- Substance P etc.

4. Others

- Purines (Adenosine & Adenosine Triphosphate)
- Nitric Oxide

Acetylcholine and Monoamines are ought to perform specialized regulating functions, often limited to explicit structures. The peptides regulate neuronal function by themselves or in concert with other neurotransmitters and execute specialized functions mainly in hypothalamus.

Table-1: Some Neurotransmitters found in Nervous system

Name	Effects	Receptor subtype	Receptor motif	Mechanism
<u>AMINES</u> Acetylcholine	Both excitatory & inhibitory	Nicotinic Muscarinic	Ionotropic Metabotropic	$\uparrow \text{Na}^+, \text{K}^+, \text{Ca}^{2+}$ conductance $\uparrow \text{IP}_3/\text{DAG}/\text{Ca}^{2+}$ $\downarrow \text{cAMP}, \uparrow \text{K}^+$ conductance

Norepinephrine	Both excitation & inhibition in the brain stem	α_1 α_2 $\beta_1, \beta_2, \beta_3$	Metabotropic Metabotropic Metabotropic	$IP_3/DAG/Ca^{2+}$ $\downarrow cAMP, \uparrow K^+, Ca^{2+}$ conductance $\uparrow cAMP$
Dopamine	Predominantly Inhibitory	D_1, D_5 D_2, D_3, D_4	Metabotropic Metabotropic	$\uparrow cAMP$ $\downarrow cAMP \uparrow K^+, \downarrow Ca^{2+}$ conductance
Serotonin (5HT)	Both excitatory & inhibitory	$5HT_1$ $5HT_2$ $5HT_3$ $5HT_{4-7}$	Metabotropic Metabotropic Ionotropic Metabotropic	$\downarrow cAMP, \uparrow K^+$ conductance $\uparrow IP_3/DAG/Ca^{2+}$ $\uparrow Na^+, K^+, Ca^{2+}$ conductance $\uparrow cAMP$
Histamine	Mainly inhibitory. Functions uncertain.	H_1 H_2 H_3	Metabotropic Metabotropic Unknown	$\uparrow IP_3/DAG/Ca^{2+}$ $\uparrow cAMP$ Unknown
Amino Acids Glycine	Inhibitory	α, β subunits	Ionotropic	$\uparrow Cl^-$ conductance
Glutamate & Aspartate	Excitatory	AMPA Kainate NMDA mGlu(1-7)	Ionotropic Ionotropic Ionotropic Metabotropic	$\uparrow Na^+, K^+$ conductance $\uparrow Na^+, K^+$ conductance $\uparrow Na^+, K^+, Ca^{2+}$ conductance $\downarrow cAMP$ $\uparrow IP_3/DAG/Ca^{2+}$
Gama-aminobutyric acid (GABA)	Inhibitory	$GABA_A$ $GABA_B$	Ionotropic Metabotropic	$\downarrow cAMP$ $\uparrow Cl^-, K^+$ conductance ^{[6][7]}

NEUROLOGIC DISORDERS

Human beings accomplish competence through multifaceted integrated processes. The major neural system which account for this competence includes; sensory, motor and cognition systems. Alteration in all of this or any one of them has an effect on competence. Some of the disorders associated with disturbances in the activity of neurological system include:

- Convulsion
- Anxiety
- Depression^[8]

CONVULSION

A **convulsion** is a medical condition where body muscle contract and relax rapidly and repeatedly, resulting in an uncontrolled shaking of the body. Because epileptic seizure is often a symptom of convulsion, the term convulsion is sometimes used as a synonym for seizure. However, not all epileptic seizure leads to convulsions are caused by epileptic seizures^[9].

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is one of the most common neurological conditions, occurring in about 1% of the global population. It is second most common disorder after stroke. Several new drugs have been licensed and many others are in various stages of development, e.g. remacemide, lamotrigine, flunarizine, loreclezole and levetiracetam. Despite optimal use of the 16 antiepileptic drugs marketed in the United States, many patients with epilepsy fail to express seizure control and others do so only at the expense of significant toxic side effects. Phenytoin, Carbamazepine and Sulfamate topiramate (TPM) are antiepileptic drugs that have been clinically effective against different types of

seizures^[10]. Estimates suggest that available medication controls the seizures in only 50% of patients or decreases the incidence in only 75% of patients. The search for antiepileptic agents with more selectivity and lower toxicity continues to be an area of investigation in medicinal chemistry. The mechanisms of action of the antiepileptic drugs (AEDs) consist in the blockade of voltage-dependent Na⁺ channels or T-type Ca²⁺ channels, inhibition of glutamatergic transmission and facilitation of γ -amino-butyric acid (GABA) inhibitory neurotransmission.^[11]

SYMPTOMS AND CAUSES

When a person is having a convulsion, they may experience several different symptoms. These may include: a brief blackout, confusion, drooling, loss of bowel/bladder control, sudden shaking of entire body, uncontrollable muscle spasms, temporary cessation of breathing, and many more. Symptoms usually last from a few seconds to around 15 minutes. If someone has a fit like this, it is advised to make sure they don't fall and injure themselves, cushion their head and loosen any restricting clothing/jewellery, and also call for medical help. Do not try to pin/hold them in place, as this could possibly cause harm or injury to the individual. Do not place anything between the person's teeth during a seizure (including your fingers).^[12]

All forms of epilepsy have their origin in the brain. The different types of epilepsies are not based on a single underlying mechanism, but are multifactorial in origin. Epilepsy results when many neurons in union, under a high excited stage, deliver massive discharges abolishing a finely organized pattern of the integrative activity of the brain. John Jackson proposed that these seizures are caused by occasional, sudden, excessive, rapid and local discharges of grey matter and once initiated by the abnormal focus, the seizures attack the neighbouring normal brain resulting into generalized convulsions. This abnormal focus may originate as a result of local biochemical changes, ischemia or the loss of vulnerable cell

inhibitory systems. However, certain physiological changes may trigger the focus and thus facilitate the spread of abnormal electrical activity to normal tissue. Such factors include

- a. Changes in blood glucose concentration
- b. Plasma pH
- c. Total osmotic pressure and electrolytes composition of extra cellular fluids
- d. Fatigue
- e. Emotional stress
- f. Nutritional deficiency
- g. Trauma, infection meningitis, brain tumors, cerebrovascular disease or metabolic abnormalities.
- h. Epileptic seizures of unidentified cause are known as primary or idiopathic epilepsy while epileptic attacks of known causes are called as secondary or symptomatic epilepsy. ^[11]

Classification Types ^[11]

[A] Generalized epilepsy: Once initiated, it spreads quickly into the entire or at least the greater part of the brain. It can be further classified into

- i. **Tonic clonic seizures** (grandmal type): It has a close resemblance with electrically induced convulsions where the mass stimulation of cortical neurons occurs. As the name indicates, initially there is a generalized tonic activity followed by clonic phase.
- ii. **Absence or minor seizures** (petitmal): It is reported to occur mainly in young children between the ages of 6 to 14 years. Seizures generally disappear spontaneously after adolescence.
- iii. **Myoclonic seizures:** The attack characterized by the jerky muscular movements of head, limbs or body as a whole. The etiology of attack is not known and is supposed to be due to brain damage.

- iv. **Infantile spasms:** The attack sometimes begins with a cry and is often associated with memory unconsciousness.

[B] Partial or focal epilepsy: In this type the initial neuronal discharge originates from a specific limited cortical area. It can further be classified as

- i. **Complex partial seizures** (psychomotor or lobe seizures); It usually originates in the anterior temporal lobe and is characterized by hallucinations, fear, hate or other emotional and behavioral abnormalities.
- ii. **Motor epilepsy:** Only one, entire side is affected, consciousness is not lost. Motor epilepsy is mainly witnessed in child hood and is due to more limited cortical abnormalities.

Mechanisms

- A. **Neuronal Sites of Action of antiepileptics** – Antiepileptic drugs acting upon neuronal site have been shown by Fig.1.
- B. **Sites of Action of antiepileptic in GABAergic Synapse** - Antiepileptic drugs acting through GABAergic Synapse have been shown by Fig.2.

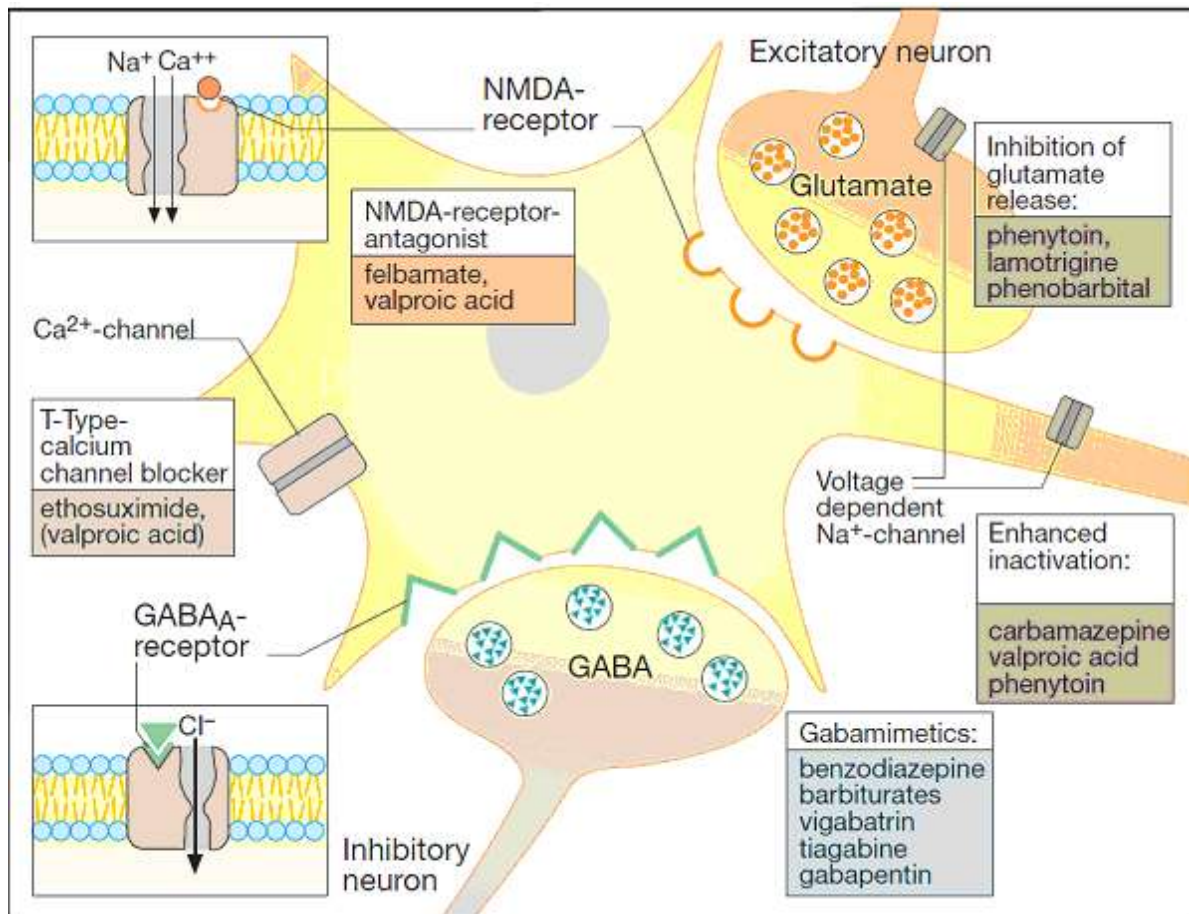


Fig 1. Neuronal sites of action of antiepileptics

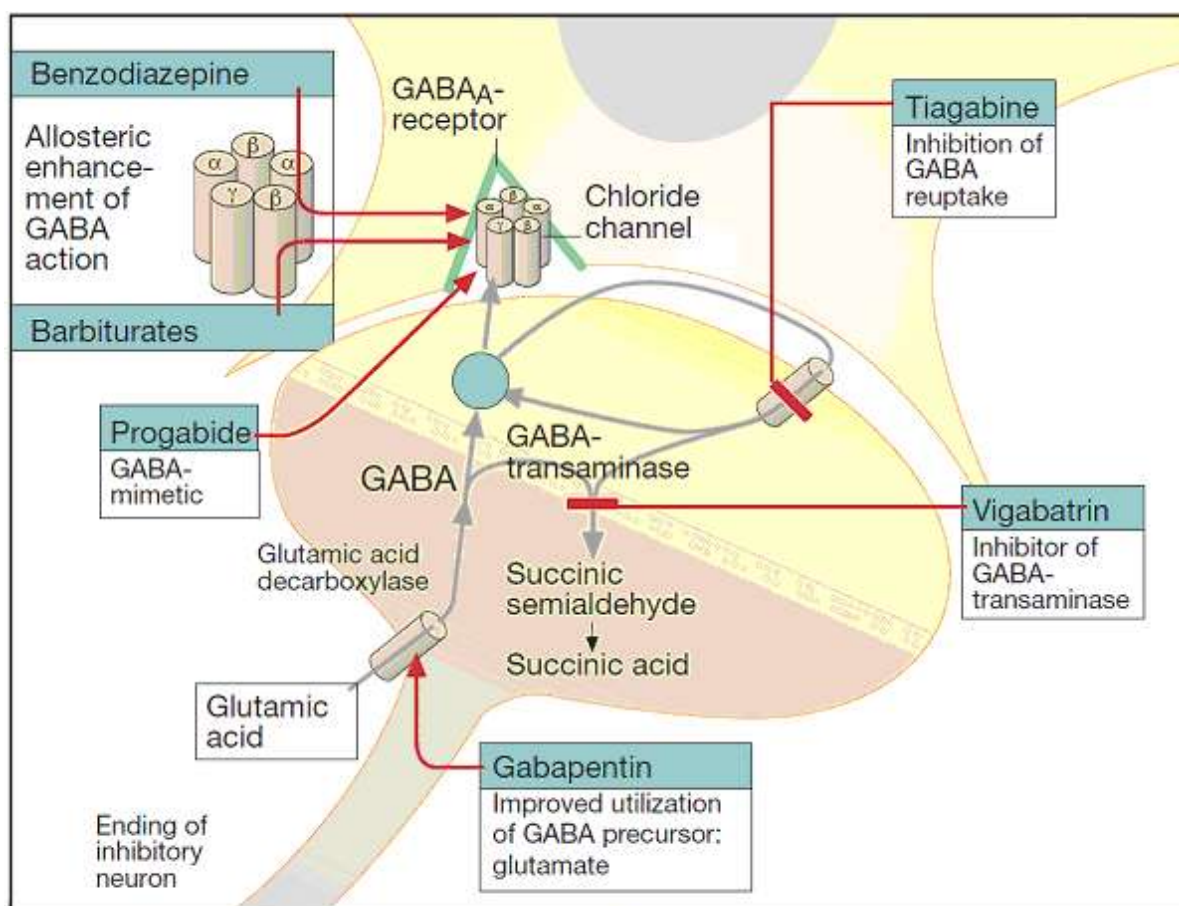


Fig.2. Sites of action of antiepileptics in GABAergic synapse

EXPERIMENTAL MODELS FOR CONVULSION

Simple Partial Seizure Models.

- i. Cortically Implanted Metals
- ii. Aluminum, Cobalt, Zinc

Complex Partial Seizure Model.

- i. Kainic Acid Administration (KA)
- ii. Repetitive Electrical Stimulation ("Kindling")

Generalized Tonic Clonic Seizure Models.

- i. Maximum electroshock
- ii. Pentylentetrazol

Generalized Partial Seizure Models.

- i. Penicillin
- ii. Bicuculine

Generalized Absence Seizure Models.

- i. Audiogenic seizures in mice.
- ii. Genetic: Photosensitive *Papio papio*

Status epilepticus.

- i. Pilocarpine

SIMPLE PARTIAL SEIZURE MODEL**Aluminium hydroxide model**

The first report on aluminium (Al) and epileptic seizures was documented by Kopeloff and collaborators in 1942, who observed that the application of alumina cream to the cerebral cortex of experimental animals resulted in the development of convulsive seizures. Later, Kopeloff and his group described a model of epileptogenesis, using aluminium hydroxide $\text{Al}(\text{OH})_3$. This was classified within the models of simple partial seizures, of the focal type. Application of 100 μL of a 3-5 % solution of $\text{Al}(\text{OH})_3$ on the cerebral cortex (Cx), causes recurring seizures after 2 months. Applying alumina cream on the motor cortex of cats Produced similar effects, showing that it can produce convulsions when applied directly. In a study in which Al gel (30-40 μL) was applied on the sensorimotor cortex, animals displayed convulsive seizures in a spontaneous manner, and this was observed for 70 days after the application of aluminium. Al is an option when studying focal experimental epilepsy, but when applied on the temporal lobe of monkeys, it can simulate a temporal lobe crisis similar to what is seen in humans. Reports indicate that this model can produce an important loss of the neuronal dendrites in the epileptic focus, as well as gliosis, therefore, an increase in the number of glial connections. Along with the cellular changes

produced by this model, there is a lower number of GABAergic neurons and a drop in the number of positive terminals for glutamate decarboxylase (GAD) around the epileptic focus. This model has been used in research for epilepsy drugs, such as diphenylhydantoin and pentobarbital, where these drugs have shown an antiepileptic effect.

Cobalt model

Cortical implantation of cobalt can produce chronic or subacute models of recurrent seizures in animals. GABA receptors have been found to be decreased in the region of cobalt foci of rat motor cortex, 2-3 weeks after establishment of the focus. Furthermore in the unilateral cobalt model, the lack of anatomic differences in the white or gray matter outside the areas of MR signal loss caused by cobalt suggests no widespread cerebral injury.

COMPLEX PARTIAL SEIZURE MODEL

Kainic acid (KA) model

In 1970, Shinozaki and Konishi showed that kainic acid (KA) Systemic administration of the appropriate dose of KA induces 'wet dog shakes', generalized tonic-clonic convulsions, teeth chattering and altered motor activity including an initial hypoactivity which transforms to a hyperactivity at later stage. Neurodegeneration occurs in the pyramidal layer of CA3 area of hippocampus and in the piriform cortex as early as 3 hours following injection. At this time point, a positive correlation exists between the dose of KA and the extent of the acute neurochemical changes including increases of 3, 4-dihydroxyphenylacetic acid and decrease in noradrenaline levels in all brain regions investigated. By 13 hours to 2 weeks, neuronal somata degenerate and disappear in areas such as the olfactory cortex and parts of the amygdaloid complex, hippocampal formation, thalamus and neocortex.

Kindling animal model:

Kindling is a model of epilepsy produced by repeated administration of an initially subconvulsive electrical or chemical stimulus that results in an increase in seizure activity, culminating in a generalized seizure. In rats the electrode is implanted in the right amygdale for electrical stimulation. Animal is allowed to recover from surgery for a minimum of 1-2 weeks; otherwise the sensitivity of the animals to kindling is lowered. Then daily electrical stimulus trains are applied via the electrode using either a fixed current strength (400-500 μ A, 1 m sec monophasic square wave pulses for 1 sec with 50 or 60/sec frequency) or using the individual threshold current to induce after discharge at the site of stimulation. During the daily electrical stimulation of amygdala, seizures develop into five stages:

Class-1: Immobility, eye closure, twitching of vibrissae, stereotypic sniffing

Class-2: Facial clonus and head nodding

Class-3: Facial clonus, head nodding and forelimb clonus

Class-4: Rearing, often accompanied by bilateral forelimb clonus

Class-5: Rearing with loss of balance and falling accompanied by generalized clonic seizures.

Rats are said to be fully kindled when enhanced sensitivity, as evidenced by class five seizures has developed. If the stimulation is continued for a few weeks, rats develop ‘spontaneous’ epileptic seizures that persists for as long as 7 months following termination of the stimulation.

Lothman et al described an alternate method for producing the fully kindled state in rats: the rapidly recurring hippocampal seizure (RRHS) model and Gupta YK et al described an alternative method for Kindling; a subconvulsive dose of PTZ (35 mg/ kg body was injected intraperitoneally on every second day for (43 days as 22 injections). The PTZ injections were stopped when the animals showed adequate kindling.

GENERALIZED TONIC CLONIC SEIZURE MODELS

Electroshock seizure model

Electrical stimulation of the brain by placing electrodes on the cornea or ears has been used to induce motor convulsions that depend on the intensity of the stimulation. Tonic-clonic convulsions are produced by high electroshock currents, between 25 mA to 150 mA and 50 Hz with duration of 2 ms. The intensity used depends on the animal, for example, in mice, a 50 mA current applied through a corneal electrode for 0.2 s, as described elsewhere, is sufficient to produce a convulsive crisis. Electrical stimulation current strengths tend to vary. Large portions of the brain are stimulated with electroshock stimulation, causing generalized neural discharges. Daily repetitive low current electroshock stimulation on a daily basis induces limbic kindling; evidence shows that responses are produced mainly in granule cells of the Hp, and also in the neocortex and pyriform cortex. Also, electroshock-induced behavioural changes persist for at least 28 days. Microinjection of GABA has an anti-convulsive effect in rats with seizures induced by electroshock. Different antiepileptic drugs were later tested using this model, such as carbamazepine and valproic acid. Lamotrigine and oxcarbazepine inhibited seizures by 50% in this model.

Pentylenetetrazol model

PTZ is a tetrazole derivative with consistent effect in a large number of animal species like mice, rats, cats, primates etc. It is believed to act by antagonizing the inhibitory GABAergic neurotransmission. PTZ test is used for screening of drugs affective in petit mal epilepsy or tonic clonic seizures. In the s.c. PTZ (or metrazol) seizure test, the convulsive dose of PTZ inducing a clonic seizure of at least 5 s duration in 97% of the animals (CD97) is subcutaneously injected and animals are observed for a postinjection period of usually 30 min for the occurrence of such a “threshold” seizure. The test is thought to be predictive of anticonvulsant drug activity against nonconvulsive (absence or myoclonic) seizures. Drug

effective in petit mal epilepsy like ethosuximide, valproic acid are effective while phenytoin, carbamazepine are not effective in the PTZ model.

GENERALIZED PARTIAL SEIZURE MODELS

Penicillin model

One of the most popular models to study simple partial seizure has been the application of 'topical' convulsants such as the antibiotic penicillin. However, penicillins have been applied in models of 'systemic focal epileptogenesis' and models of generalized tonic-clonic and absence seizure. The convulsive properties of penicillin were first observed by Walker and Johnson,

When penicillin is applied topically to exposed rat or cat cortex through a cottonoid pledget (soaked in 1.7-3.4 mM penicillin), acute focal seizures develop. Regionally placed electrodes record recurring interictal spikes that resemble human interictal spikes within a few minutes of application. Initial descriptions of the 'paroxysmal depolarisation shift', considered to be a hallmark of epilepsy, were based upon intracellular recordings from penicillin foci in rat neocortex. This model has also been considered suitable for study of spread of seizure activity.

Parental administration of penicillins produces generalized seizures in cats and mice. Seizures induced by parenteral penicillin in rat bear little resemblance of clinical absences. However, parenteral penicillin G administration ($\geq 300,000$ units intramuscular (i.m)/kg) in cat results in recurrent episodes of arrested activity, starting, myoclonus, etc. While diazepam, carbamazepine and phenytoin have a high protective efficacy, phenol-barbital has intermediate effectiveness; the antiabsence agents, valproate and ethosuximide, display a low effectiveness.

Gaba abstinence model

This model was originally described by Snead and is a focal epilepsy model, which can be induced by injecting GABA in the motor cortex. It is known that animals receiving chronic treatment with this amino acid, and then had it removed; display an electrocorticographic activity pattern of epilepsy. In this model, peaks of activity are displayed in the waves of the EEG, from 200 to 700 μ V, with frequencies from .05 to 3 cycles per second in the motor cortex, followed by mioclonic automatisms of the fore and hind limbs. According to previous descriptions, increases in the concentration of glutamate antagonists are accompanied by neurotoxic effects.

GENERALIZED ABSENCE SEIZURE MODEL**Audiogenic seizure model**

Audiogenic seizure-prone mice have proved useful for identifying potential AEDs. When these mice are exposed to high-frequency (10–20 kHz) and high intensity (90–120 dB) sound, they exhibit wild running, followed by generalized clonic-tonic seizures. With some seizure-susceptible strains of mice, the seizure focus must be established with a priming stimulus of high intensity sound. In several strains of mice, seizure susceptibility changes with age, usually reaching a maximal level between 2 and 4 weeks of life; however, the Frings and O'Grady strains of mice may retain their susceptibility to seizures into their adult life. The most commonly used strain of audiogenic seizure-prone mice for anticonvulsant identification is the DBA/2J. It is an inbred strain of the house mouse (*Mus musculus*), is the most studied strain of audiogenic seizure-susceptible mice.

STATUS EPILEPTICUS**Lithium-pilocarpine model**

Pilocarpine hydrochloride is an agonist of muscarinic Ach receptors expressed in the Hp, and is known to produce seizures by increasing the activation of these receptors. One

recently popularized model of status epilepticus is the lithium-pilocarpine model. In this model rats are pretreated with lithium chloride. At least 20 h later the cholinergic agent pilocarpine is given. Generalized clonic or tonic-clonic seizure activity begins about 30 min after administration of pilocarpine, and continues for several hours. The EEG pattern displays a progression very similar to the stages seen in human status epilepticus. Chronic pretreatment for one month with daily lithium reduces the convulsant threshold of pilocarpine 26-fold. In lithium-pilocarpine treated adult rats, neuronal damage and neuronal death develops mainly in the hippocampus, the hilus of the dentate gyrus, the piriform and entorhinal cortices, the amygdala, the neocortex and the thalamus. Also, lithium-pilocarpine treatment leads to hippocampal damage that is typically observed in the CA1 and CA3 pyramidal cell layers and the hilus of the dentate gyrus in mice with status epilepticus.^[13]

ANTI-CONVULSANT

CLASSIFICATION OF ANTI-CONVULSANT DRUGS

1) Action on Ion Channels

Sodium channels:

- i. Phenytoin
- ii. Carbamazepine
- iii. Topiramate
- iv. Valproic acid

Calcium channels:

- i. Ethosuximide
- ii. Valproic acid

2) Enhance GABA Transmission

- Benzodiazepines
 - i. Diazepam

- ii. Clonazepam
- iii. Lorazepam
- Barbiturates
 - i. Valproic acid
 - ii. Gabapentin
 - iii. Vigabatrin
 - iv. Topiramate
 - v. Felbamate

3) Inhibit EAA Transmission

- i. Felbamate
- ii. Topiramate

CLASSIFICATION OF CLASSICAL / NEWER :

Classical

- i. Phenytoin
- ii. Phenobarbital
- iii. Topiramate
- iv. Tiagabine
- v. Vigabatrin
- vi. Oxycarbazepine

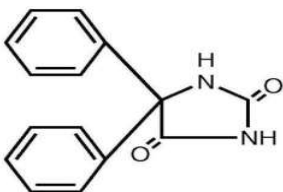
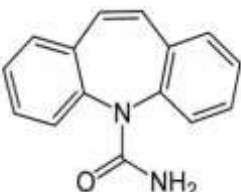
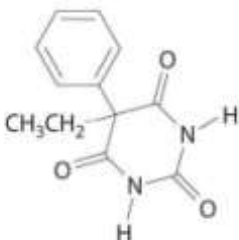
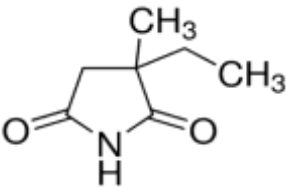
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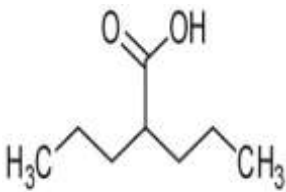
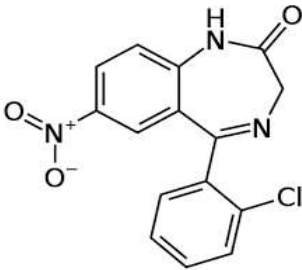
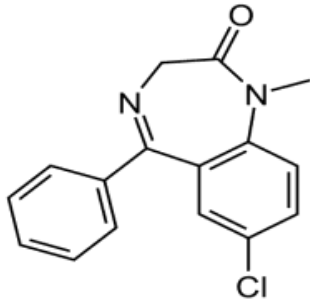
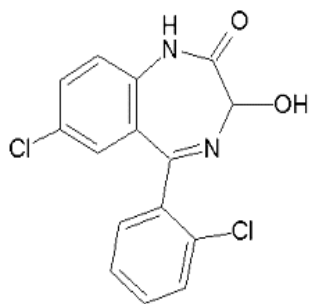
- i. Lamotrigine
- ii. Primidine
- iii. Carbamazepines
- iv. Ethosuximide
- v. Valproic acid
- vi. Trimethadione
- vii. Levetiracetam
- viii. Fosphenytoin

Established & Newer Antiepileptic Drugs

The currently used therapy of epilepsy includes various drugs that act through different mechanisms. There are several classes of antiepileptic drugs (AEDs) that have been divided on the basis of their chemical structure. Different established AEDs and newer antiepileptic drugs along with the drugs in pipeline are summarized in Table 1 and 2.

Table-2: General Description of Established Antiepileptic Drugs

Drug	Structure	Clinical Uses	Mechanism
Phenytoin (Dilantin)		Partial and tonic-clonic	Prolongs closing of inactivating gate of sodium channels of excitatory NT receptors in the CNS
Carbamazepine (Tegretol)		Partial and tonic-clonic	Prolongs closing of inactivating gate of sodium channels of excitatory NT receptors in the CNS
Phenobarbital (Luminal®)		Partial and tonic-clonic	Facilitates the inhibitory action of GABA, increases the duration of chloride channel opening at GABA-A receptors
Ethosuximide (Zarontin®)		Absence seizures	Inhibits low-threshold T-type calcium currents in thalamic neurons

Valproic acid (Depakene®) Divalproex Na (Depakote®)		Partial and tonic-clonic and absence seizures	Prolongs inactivation of sodium channels of excitatory NT receptors in CNS Inhibits low-threshold T-type calcium currents in thalamic neurons. Increases the amount of GABA in CNS. Increases GAD activity. Decreases GABA-T and succinic semialdehyde dehydrogenase activity
Clonazepam (Klonopin®)		Absence and myoclonic	Facilitates the inhibitory actions of GABA
Diazepam (Valium®)		Status epilepticus	Increases the frequency of opening of chloride channel of GABA-A receptor
Lorazepam (Ativan®)		Status epilepticus	Increases the frequency of opening of chloride channel of GABA-A receptor

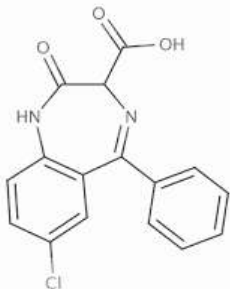

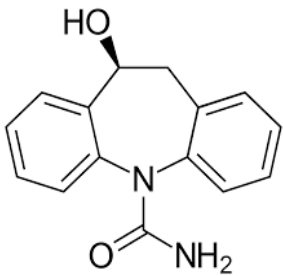
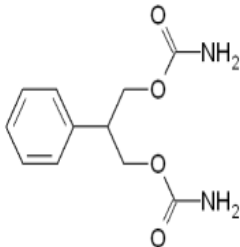
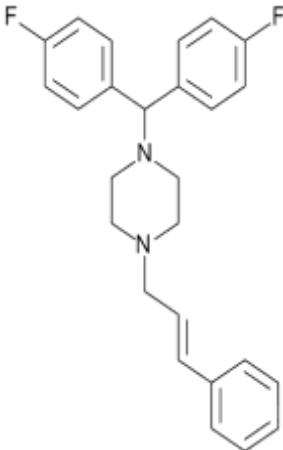
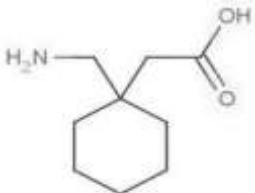
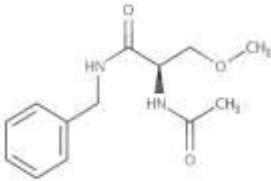
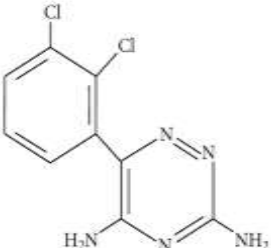
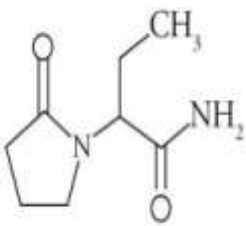
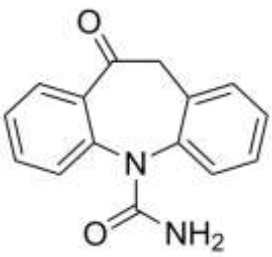
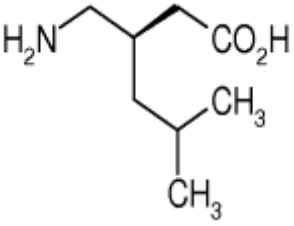
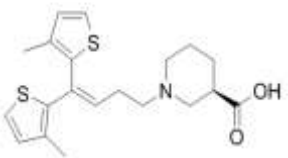
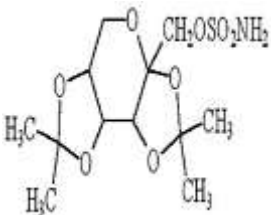
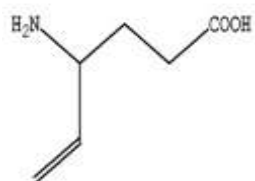
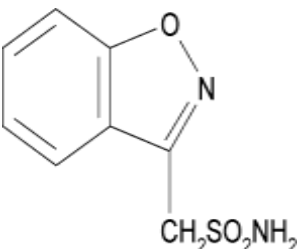
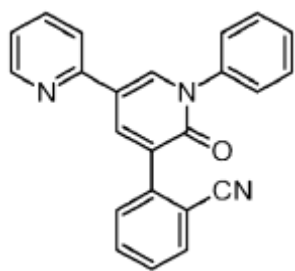
Chlorazepate (Tranxene®)		Partial myoclonic, absence.	Increases the frequency of opening of chloride channel of GABA-A receptor
Trimethadione		Absence	Inhibits low-threshold T- type calcium currents in thalamic neurons
Bromide	Br -	Epilepsy in porphyrias.	Not known

Table-3: General Description of New Anticonvulsants**A) Marketed Drugs**

DRUGS	STRUCTURE	MECHANISM	CLINICAL USES	SIDE EFFECTS
Eslicarbazepine (Zebinix; Exalife)		Novel voltage gated Na ⁺ - channel blocker	As adjunct in partial onset seizures;also in bipolar disorders and trigeminal neuralgia	-
Felbamate (Felbatrol)		Possible blockade of NMDA receptor	Partial seizures lennox-gastaut syndrome	Server hepatitis aplastic anemia

Flunarizine (Sielium)		Ca ⁺⁺ - Channel blocker with calmodulation binding property and Histamine blocking activity	-	-
Gabapentin (Neurontin)		Increase the release of GABA	Adjunct drug for partial and generalized tonic-clonic seizures	Somnolence dizziness Ataxia Headache
Lacosamide (Vimpat)		Enhances slow activation voltage gated Na ⁺ Channel	-	-
Lamotrigine (Lamictal)		Prolongs closing of inactivating gate of Na ⁺ channel	Partial seizures	Dizziness headache diplopia somnolence skin rashes

Levetiracetam (Keppra)		Binds to synaptic vesicles protein SV2A thereby impeding nerve conduction across synapse	Adjunct for partial seizures with or without secondary generalization	Minimal drowsiness Anxiety Amnesia
Oxcarbazepine (Trileptal)		Blockade of voltage sensitive sodium channels	Partial seizures with or without generalization	CNS side effects, hematological abnormalities and effects on drugs metabolizing enzymes are less than carbamazepines
Pregabalin (Lyrica)		Not Known	For neuropathic pain and adjunct therapy for partial seizures	-
Tiagabine (Gabatril)		Inhibition of GABA uptake	Adjunct for partial seizure	Nervousness Dizziness Tremors Depression

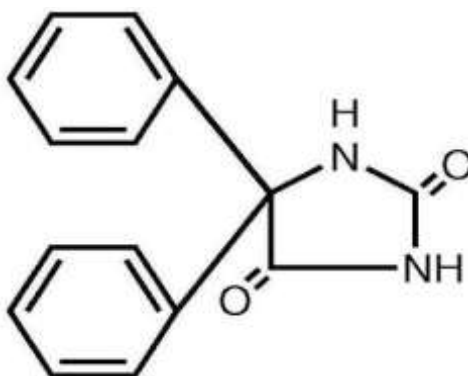
Topiramate (Topamax)		Prolongs closing of inactivating gate of Na^+ channel, potentiates the GABA effects and blocks AMPA receptors	Partial seizures	Drowsiness Parathesia Confusion
Vigabatrin (sabril)		Irreversible inhibitor of GABA aminotransferase (GABA-T)	Partial seizures	Drowsiness Dizziness Weight gain Psychosis
Zonisamide (Zonegran)		Inactivation of Na^+ And Ca^{++} channels	Partial and generalized tonic-clonic seizures	Drowsiness Cognitive Impairment
Perampanel (E2007)		Selective antagonist for the AMPA sub type of ionotropic glutamate receptors	Drug suggests efficacy and safety in refractory epilepsy	Dizziness Drowsiness Irritability Headache Falls Ataxia ^[11]

PHENYTOIN :

Phenytoin,(Dilantin) , is an anti-seizure medication. It is useful for the prevention of tonic-clonic seizures, partial seizures, but not absence seizures. The intravenous form is used for status epilepticus that does not improve with benzodiazepines. It may also be used for certain heart arrhythmias or neuropathic pain. It can be taken intravenously or by mouth. The intravenous form generally begins working within 30 minutes and is effective for 24 hours. Blood levels can be measured to determine the proper dose.

Structure of phenytoin

(5,5-DIPHENYHYDANTOIN)



Common side effects include nausea, stomach pain, loss of appetite, poor coordination, increased hair growth, and enlargement of the gums. Potentially serious side effects include sleepiness, self harm, liver problems, bone marrow suppression, low blood pressure, and toxic epidermal necrolysis. There is evidence that use during pregnancy results in abnormalities in the baby. It appears to be safe to use when breastfeeding. Alcohol may interfere with the medication's effects.

MECHANISM OF ACTION

Phenytoin is believed to protect against seizures by causing voltage-dependent block of voltage gated sodium channels. This blocks sustained high frequency repetitive firing of action potentials. This is accomplished by reducing the amplitude of sodium-dependent action potentials through enhancing steady state inactivation. Sodium channels exist in three main conformations: The resting state, the open state, and the inactive state

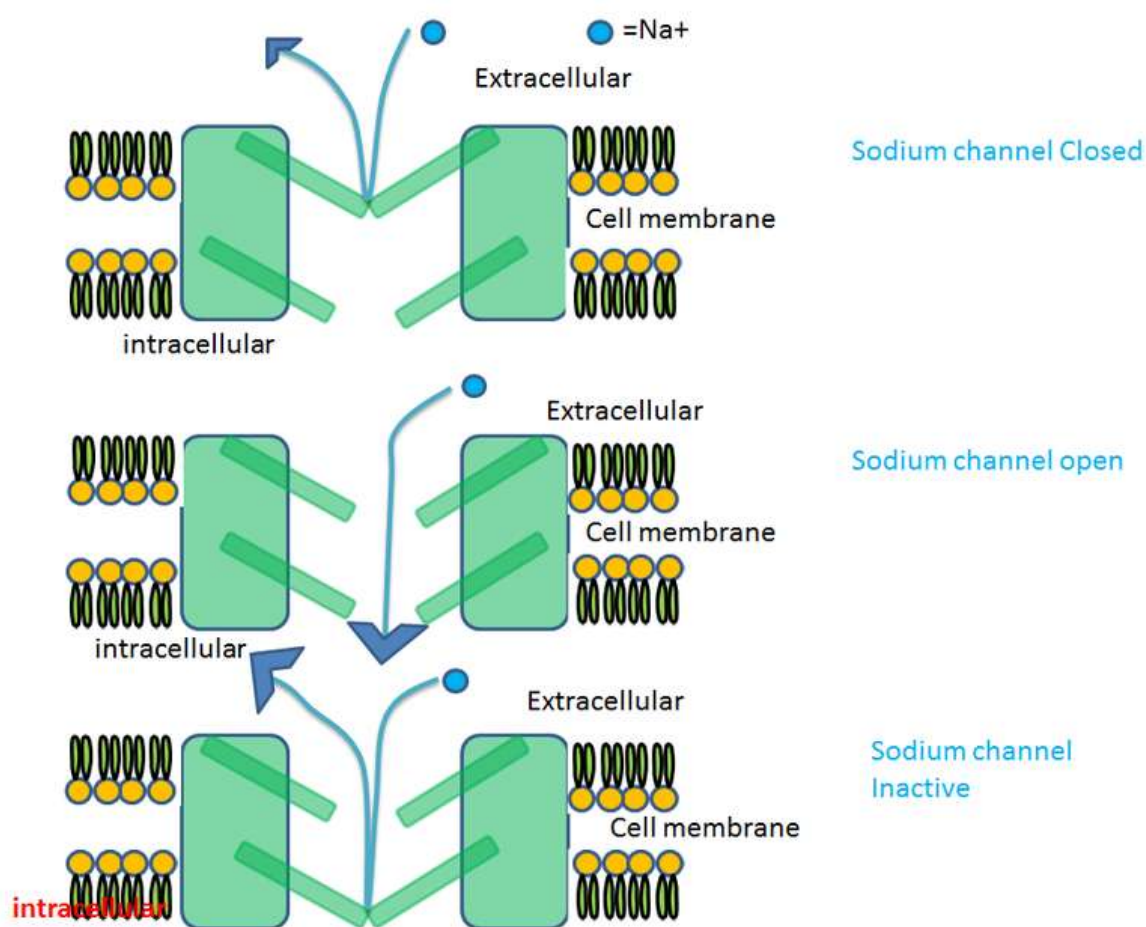


Fig-3 Mechanism of action Phenytoin

Phenytoin binds preferentially to the inactive form of the sodium channel. Because it takes time for the bound drug to dissociate from the inactive channel, there is a time dependent block of the channel. Since the fraction of inactive channels is increased by

membrane depolarization as well as by repetitive firing, the binding to the inactive state by phenytoin sodium can produce voltage-dependent, use-dependent and time-dependent block of sodium-dependent action potentials.

The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited. Possibly by promoting sodium efflux from neurons, phenytoin tends to stabilize the threshold against hyper excitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient. This includes the reduction of post-tetanic potentiating at synapses which prevents cortical seizure foci from detonating adjacent cortical areas. Phenytoin reduces the maximal activity of brain stem centers responsible for the tonic phase of generalized tonic-clonic seizures.

Pharmacokinetics

Phenytoin elimination kinetics show mixed-order behaviour at therapeutic concentrations. A small increase in dose may lead to a large increase in drug concentration as elimination becomes saturated. The time to reach steady state is often longer than 2 weeks.

Therapeutic uses

Seizures

- a) **Tonic-clonic seizures:** Mainly used in the prophylactic management of tonic-clonic seizures with complex symptomatology (psychomotor seizures). A period of 5–10 days may be required to achieve anticonvulsant effects.
- b) **Focal seizures:** Mainly used to protect against the development of focal seizures with complex symptomatology (psychomotor and temporal lobe seizures). Also effective in controlling partial seizures with autonomic symptoms.
- c) **Absence seizures:** Not used in treatment of pure absence seizures due to risk for increasing frequency of seizures. However, can be used in combination with other anticonvulsants during combined absence and tonic-clonic seizures.

- d) **Seizures during surgery:** Used as prevention and treatment of seizures occurring during and after neurosurgery.
- e) **Status epilepticus:** Considered after failed treatment using a benzodiazepine due to slow onset of action.

Other:

f) **Abnormal heart rhythms:** may be used in the treatment of ventricular tachycardia and sudden episodes of atrial tachycardia after other antiarrhythmic medications or cardioversion has failed. It is a class 1b antiarrhythmic.

Special considerations:

Monitoring plasma concentrations:

- g) **Pregnancy:** Pregnancy Category D due to risk of fetal hydantoin syndrome and fetal bleeding. However, optimal seizure control is very important during pregnancy so drug may be continued if benefits outweigh the risks. Due to decreased drug concentrations during pregnancy, dose of phenytoin may need to be increased if only option for seizure control.
- h) **Breast feeding:** The manufacturer does not recommend breast feeding because low concentrations of phenytoin are excreted in breast milk .
- i) **Liver disease:** Do not use oral loading dose. Consider using decreased maintenance dose.
- j) **Kidney disease:** Do not use oral loading dose. Can begin with standard maintenance dose and adjust as needed.^[14]

DEPRESSION

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. These problems can become chronic or recurrent and lead to

substantial impairments in an individual's ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide, a tragic fatality associated with the loss of about 850 000 lives every year. Depression is the leading cause of disability as measured by YLDs (Years Lived with Disability) and the 4th leading contributor to the global burden of disease (DALYs- Disability Adjusted Life Years) in 2000. By the year 2020, depression is projected to reach 2nd place of the ranking of DALYs calculated for all ages, both sexes. Today, depression is already the 2nd cause of DALYs in the age category 15-44 years for both sexes combined. Depression occurs in persons of all genders, ages, and backgrounds.

DIAGNOSIS

Currently, no laboratory test can be used to diagnose depression. Depression is diagnosed based on your reported symptoms, signs that your doctor observes while interviewing you, your medical history and your family's medical history. Criteria outlined in a handbook called the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) are used in making the diagnosis. According to the *DSM-IV*, a person who suffers from major depressive disorder must either have a depressed mood or a loss of interest or pleasure in daily activities consistently for at least a two week period. This mood must represent a change from the person's normal mood; social, occupational, educational or other important functioning must also be negatively impaired by the change in mood. A depressed mood caused by substances (such as drugs, alcohol, medications) or which is part of a general medical condition is not considered to be major depressive disorder. Major depressive disorder cannot be diagnosed if a person has a history of manic, hypomanic, or mixed episodes (e.g., a bipolar disorder) or if the depressed mood is better accounted for by schizoaffective disorder and is not superimposed on schizophrenia, schizophreniform disorder, delusional disorder or psychotic disorder.

SYMPTOMS AND SIGNS

The symptoms should not be accounted for by another illness, drugs of abuse or prescription medications. Common symptoms of depression are as follows:

A. Depressed Mood:

- A person may report feeling "sad" or "empty" or may cry frequently. Children and adolescents may exhibit irritability.

B. Decreased Interest or Pleasure:

- A person may show markedly diminished interest or pleasure in all, or almost all, daily activities.

C. Weight Changes:

- Significant changes in weight when not attempting to gain or lose (a gain or loss of 5% or more in a month) may be indicative of depression. In children, this may also present as a failure to make expected weight gains.

D. Sleep Disturbances:

- Insomnia or sleeping too much may be a symptom of depression.

E. Psychomotor Agitation or Retardation:

- The person may be observed to be either agitated or restless or physically slowed down in their movements.

F. Fatigue:

- Deep fatigue or a loss of energy is a symptom of depression.

G. Feelings of Worthlessness or Guilt:

- A depressed person may feel that they have no value or they may feel inappropriately guilty about things they have no control over.

H. Brain Fog:

- A depressed person may have a diminished ability to think, concentrate or make decisions.

CAUSES OF DEPRESSION

The causes of depression are complex. Genetic, biological, and environmental factors can contribute to its development. In some people, depression can be traced to a single cause, while in others; a number of causes are at play. For many, the causes are never known. Currently, it appears that there are biochemical causes for depression, occurring as a result of abnormalities in the levels of certain chemicals in the brain. While we still don't know exactly how levels of these neurotransmitters affect mood, we do know that the levels can be affected by a number of factors. Monoamine hypothesis of depression has been shown in Fig. 4

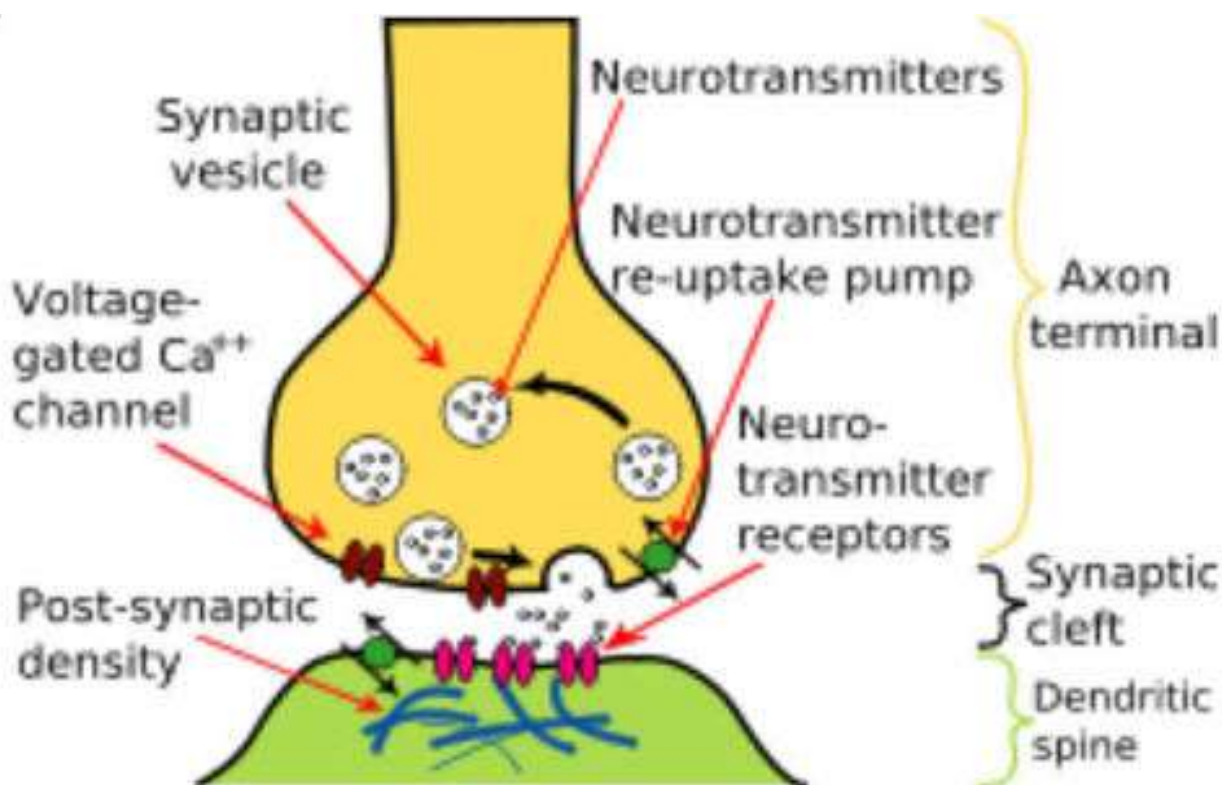


Fig-4 Monoamine hypothesis of depression

TYPES OF DEPRESSION

There are several different types of clinical depression (mood disorders that include depressive symptoms):

A. Major depression:

It is an episode of change in mood that lasts for weeks or months. It is one of the most severe types of depression. It usually involves a low or irritable mood and/or a loss of interest or pleasure in usual activities. It interferes with one's normal functioning and often includes physical symptoms. A person may experience only one episode of major depression, but often there are repeated episodes over an individual's lifetime.

B. Dysthymia:

It is less severe than major depression but usually goes on for a longer period, often several years. There are usually periods of feeling fairly normal between episodes of low mood. The symptoms usually do not completely disrupt one's normal activities.

C. Bipolar disorder:

It involves episodes of depression, usually severe, alternating with episodes of extreme elation called mania. This condition is sometimes called by its older name, manic depression. The depression that is associated with bipolar disorder is often referred to as bipolar depression. When depression is not associated with bipolar disorder, it is called unipolar depression.

D. Seasonal depression:

Which medical professionals call seasonal affective disorder, or SAD, is depression that occurs only at a certain time of the year, usually winter, when the number of daylight hours is lower. It is sometimes called "winter blues." Although it is predictable, it can be very severe.

E. Psychotic depression:

It refers to the situation when depression and hallucinations or delusions are experienced at the same time (co-occur). This may be the result of depression that becomes so severe that it results in the sufferer losing touch with reality. Individuals who primarily suffer from a loss of touch with reality (for example, schizophrenia) are thought to suffer from an imbalance of dopamine activity in the brain and to be at risk of subsequently becoming depressed.^[15]

CLASSIFICATION

There are many antidepressant drugs available in the market. The key role is played by the time that might be required for a particular outcome of the drug on the individual i.e. the response time of a drug can be known in the due course. The commonly used antidepressants,

- Selective serotonin re-uptake inhibitors (SSRIs)
 - i. fluoxetine
 - ii. sertraline
 - iii. citalopram
 - iv. Escitalopram
 - v. paroxetine
 - vi. fluvoxamine
- Serotonin and norepinephrine re-uptake inhibitors (SNRIs)
 - i. Venlafaxine
 - ii. Desvenlafaxine
 - iii. Duloxetine
 - iv. Milnacipran

- Monoamine oxidase inhibitors (MAOIs)
 - i. Phenelzine
 - ii. Isocarboxazid
 - iii. Tranylcypromine
 - iv. Selegiline
 - v. Moclobemide
- Tricyclic antidepressants (TCAs)
 - i. imipramine
 - ii. clomipramine
 - iii. amitriptyline
 - iv. desipramine
 - v. trimipramine
 - vi. nortriptyline
 - vii. protriptyline
 - viii. doxepin
- Tetracyclic antidepressants
 - i. Amoxapine
 - ii. Maprotiline
 - iii. Bupropion
 - iv. Mirtazapine
- Lithium Salts^[16]

Mechanism of Action

Neurotransmitters are endogenous chemicals that transmit signals across a synapse from one neuron to another 'target' neuron. Brain neurotransmitters might not be secreted in adequate amounts to alleviate mood disorders. The chemicals like serotonin, melatonin, and

dopamine are the most important in brain for sense. Once the nerves are robbed of those neurotransmitters, they can't send messages to different nerves which leads to depression. The messages that are passed through the neurons are exhibited as emotions, behavior, temperature, appetite, or several alternative functions. The information sent depends on that neurons area unit activated and what a part of the brain is excited.

Low levels of serotonin and norepinephrine within the conjunction area leads to depression. Hence medications like antidepressant used to treat this works by increasing the number of bound neurotransmitters in that particular part of the brain which enables to transmit the message.

Each type of antidepressant works on brain with little difference, all antidepressant medications influence the neurotransmitters how to work in the brain, especially serotonin and norepinephrine, thus controlling the balance of the neurotransmitters.

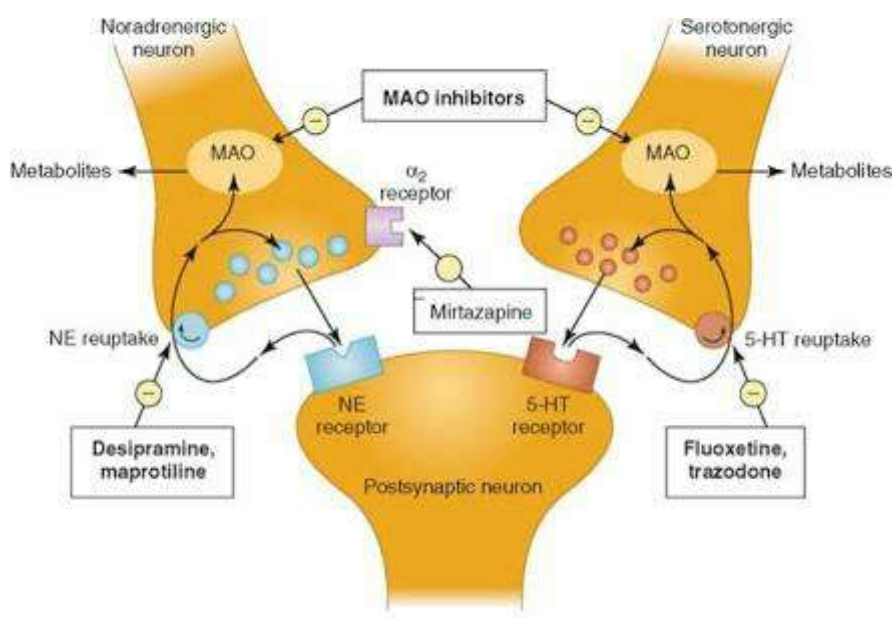


Fig-5 general mechanism of action anti depressant

SSRIs (selective serotonin reuptake inhibitors) and SNRIs (serotonin norepinephrine reuptake inhibitors) have different mechanisms of action. SSRI has three different serotonin reuptake inhibitors those are fluoxetine, paroxetine and sertraline. These have selective effect

for both citalopram and fluvoxamine on the serotonin reuptake pump. It leads to primary increase in serotonin at the cell body and dendrites. So, SSRIs act by blocking the serotonin reuptake pump (5-HTT). Whereas SNRIs presumably block both 5-HTT and the norepinephrine transporter (NET). Blocking these transporters prevents the neuron from vacuuming up excess neurotransmitters, permitting a lot of to stay within the synapse and stimulate postsynaptic receptors. SSRIs have important effect on NE as well, and the SNRIs behave much more like SSRIs.

We extend levels of all 3 monoamines (serotonin, monoamine neurotransmitter, and dopamine), ought to be prescribing MAOIs (monoamine enzyme inhibitors). MAOIs aren't re-uptake blockers at all; they increase neurotransmitter levels by inhibiting MAO, associate enzyme that breaks down all 3 monoamines. Thus, MAOIs increase the amount of all 3 neurotransmitters thought crucial in depression, these are efficacy advantage over others.

Tricyclic and tetracyclic antidepressants ease depression by affecting naturally occurring chemical messengers. Cyclic antidepressants usually block the results of 2 neurotransmitters known as serotonin and norepinephrine these are available in the brain. This looks to assist brain cells send and receive messages. The roles these chemicals have treat the depression.

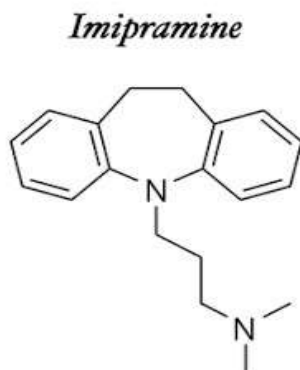
Serotonin receptor modulators utilized in the treatment of irritable intestine syndrome. Serotonin plays a major role within the initiation of peristaltic and humour reflexes, and in modulation of visceral sensations.

Lithium is used for manic depression. Manic-depressive patients expertise severe mood changes, starting from associate degree excited or frenzied state to depression or unhappiness .

Until higher models of depression area unit devised, establishing the mode of action of antidepressants are trouble. The present focus has been on alterations to straightforward

vegetative cell models primarily based around monoamine neurotransmitter and vasoconstrictive. Clearly, these models don't seem to be sufficient to fully make a case for the clinical effects of antidepressants. Additional complicated models, taking under consideration alternative transmitters, adaptive changes at the amount of the sequence^[17]

IMIPRAMINE (Structure)



Mechanism of action

Tricyclic antidepressants (TCAs) are a group of drugs used to treat affective, or 'mood', disorders. Despite being an important group of antidepressant drugs they are not ideal, due to a number of unwanted side effects. Side effects of the TCAs include sedation, caused by histamine H₁ receptor blockade; postural hypotension, due to α adrenoreceptor blockade; and blurred vision, dry mouth and constipation, due to muscarinic acetylcholine receptor blockade.^[18]

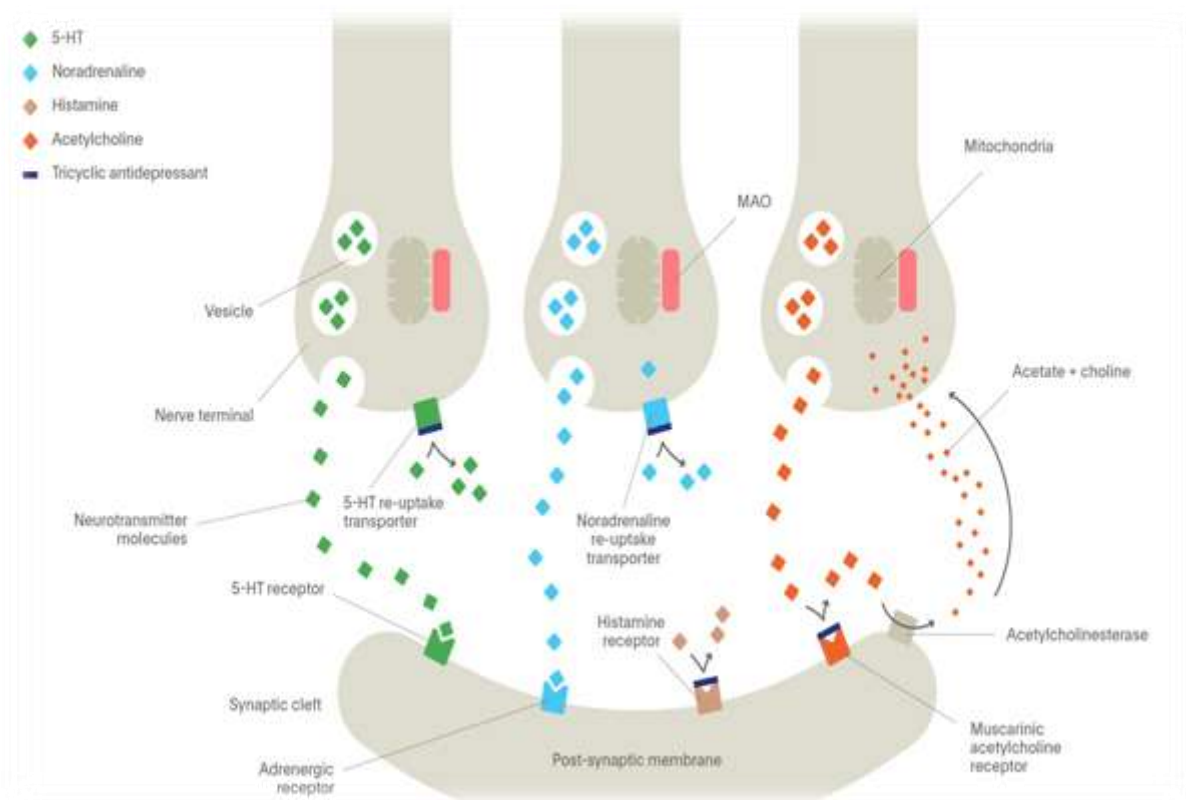


Fig-6 Mechanism of action TCAs

Pharmacokinetics

The TCA drugs are well absorbed from the gastrointestinal tract, are extremely lipid soluble, and bind extensively to plasma proteins. Their half-lives range from 8 to 89 hours. Several days to weeks are required both to achieve steady-state serum levels and for complete elimination of these agents from the body. Long half-lives make most of these agents amenable to dosing once a day, generally at bedtime. Drug inactivation generally occurs through oxidative metabolism by hepatic microsomal enzymes. Tertiary amines are converted to secondary amines, which generally possess biological activity and are frequently in serum at levels equal to or greater than that of the parent tertiary amine. A second route of inactivation includes conjugation of hydroxylated metabolites with glucuronic acid^[19].

Medical uses**Depression Mild to moderate:**

Normally advised psychotherapy, cognitive, behavior therapy. If depression is more, advised antidepressants.

If acute therapy is required, effects of antidepressants are not seen for 1-2 months. A trial of 2-3 months has to be given. If no effects appear after 3 months, doctor may switch to another one or a combination. After patient is stabilized, treatment is given for at least 6-12 months. If not given for long period, there are higher chances especially of relapse. At times recurrences of attacks are seen in sensitive patients.

Maintenance therapy is prescribed for longer duration. Studies have not been done for more than 5 years.

In some forms of bipolar disorders, antidepressant use is controversial. In this type, if antidepressant is taken, patient may go into mania. Its better to give mood stabilizing agent. Choice of antidepressant depends on severity of disease and patient's profile.^[20]

ANXIETY

Anxiety may be defined as an unpleasant state of mental uneasiness, apprehension, nervousness and obsession or concern about something uncertain. The major symptoms include; arousal, tenseness, increased autonomic activity like respiration, blood pressure and heart rate. Tightness in chest, palpitations, perspirations etc. it is a common symptom in a variety of distinct mental illnesses and is a predominant symptom in panic disorders, phobias and obsessive compulsive disorder.

MAJOR TYPES OF ANXIETY INCLUDE:

Panic disorder: Psychiatric condition associated with multiple disabling panic attacks. In between the panic attacks, an excessive time is spent by the individual in thinking about future panic attacks.

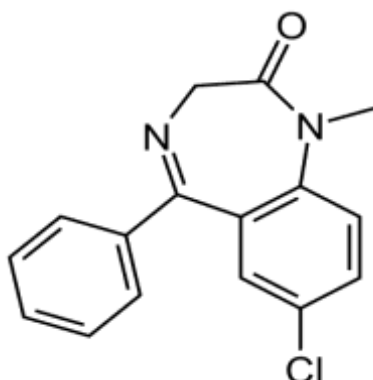
Generalized anxiety disorder: It is characterized by persistent and excessive worries. The patient worries about various life events such as job performances, marital status, money, social status etc.

Posttraumatic stress disorder: Caused due to the exposure of individual to life-threatening or terrifying life events. The individual re-experiences the traumatic event as flashbacks or as intrusive recollections.

Obsessive – compulsive disorders: The root cause of the disorder is intrusive, repetitive compulsions and / or thoughts. Marked distress is the hallmark of this disorder. There occur irrational thoughts and acts which impair normal functioning.

Classification of Anti-Anxiety

- Benzodiazepines:
 - i. Diazepam
 - ii. Lorazepam
 - iii. Alprazolam
- Azapironees:
 - i. Buspirone
- Sedative antihistaminic:
 - i. Hydroxyzine
- Beta blocker:
 - i. Propranolol.^{[21][22]}

Benzodiazepines(BZD)**Diazepam (Structure)**

Diazepam were Introduced round 1960 as antianxiety drugs. Since then class has proliferated and has gained popularity over barbiturates as hypnotic and sedative as well, because-

1.BZDs have a high therapeutic index. Ingestions 20 hypnotic doses does not usually endangers life-there is no loss of consciousness through amnesia occurs) and patient can be respiration is not so depressed as to assistance.

2. Hypnotic doses do not affect respiration or cardiovascular functions. Higher doses produce mild respiratory depression and hypotension which is problematic only in patients with respiratory insufficiency and cardiac/haemo- dynamic abnormality.

3.BZDs have practically no action on others body system . Only on i.v. injection the BP falls May be marked in an occasional patient) and cardiac contractility decreases. Fall in BP in diazepam and lorazepam is due to reduction in cardiac output while that due to midazolam is due to decrease in peripheral resistance. The coronary arteries dilate on i.v. injection of diazepam.

4.BZDs cause less distortion of sleep architecture rebound phenomena on discontinuation of regular use are less marked.

5.BZDs do not alter disposition of other drugs microsomal enzyme induction.

6.They have lower abuse liability: tolerance is mild psychological and physical dependence and withdrawal syndrome are less marked.

7.A specific BZD antagonist flumazenil is available which can be used in case of poisoning.

Mechanism of action

Benzodiazepines act preferentially on midbrain ascending reticular formation (which maintains wakefulness) and on limbic system (thought and mental functions). Muscle relaxation is produced by a primary medullary site of action and ataxia is due to action on cerebellum. BZDs act by enhancing presynaptic/postsynaptic inhibition through a specific BZD receptor which is an integral part of the GABA_A receptor-Cl⁻ channel complex. The subunits of this complex form a pentameric transmembrane anion channel (Fig.6) gated by the primary ligand (GABA), and modulated by secondary ligands which include BZDs. Only the α and β subunits are required for GABA action, and most likely the binding site for GABA is located on the β subunit, while the α/β subunit interface carries the BZD binding site. The modulatory BZD receptor increases the frequency of Cl⁻ channel opening induced by sub maximal concentrations of GABA. The BZDs also enhance binding of GABA to GABA_A receptor.

The GABA_A antagonist bicuculline antagonizes BZD action in a non competitive manner. It is noteworthy that the BZDs do not themselves increase Cl^- conductance; have only GABA facilitatory but no GABA mimetic action. This probably explains the lower ceiling CNS depressant effect of BZDs. The BZD receptor exhibits a considerable degree of constitutive activation. As such, it is capable of fine tuning GABA action in either direction. While the BZD-agonists enhance GABA induced hyperpolarisation (due to influx of Cl^- ions), and decrease firing rate of neurones, other compounds called BZD-inverse agonist like dimethoxyethyl-carbomethoxy-beta-carboline (DMCM) inhibit GABA action and are

convulsants. The competitive BZD-antagonist flumazenil blocks the sedative action of BZDs well as the convulsant action of DMCM.

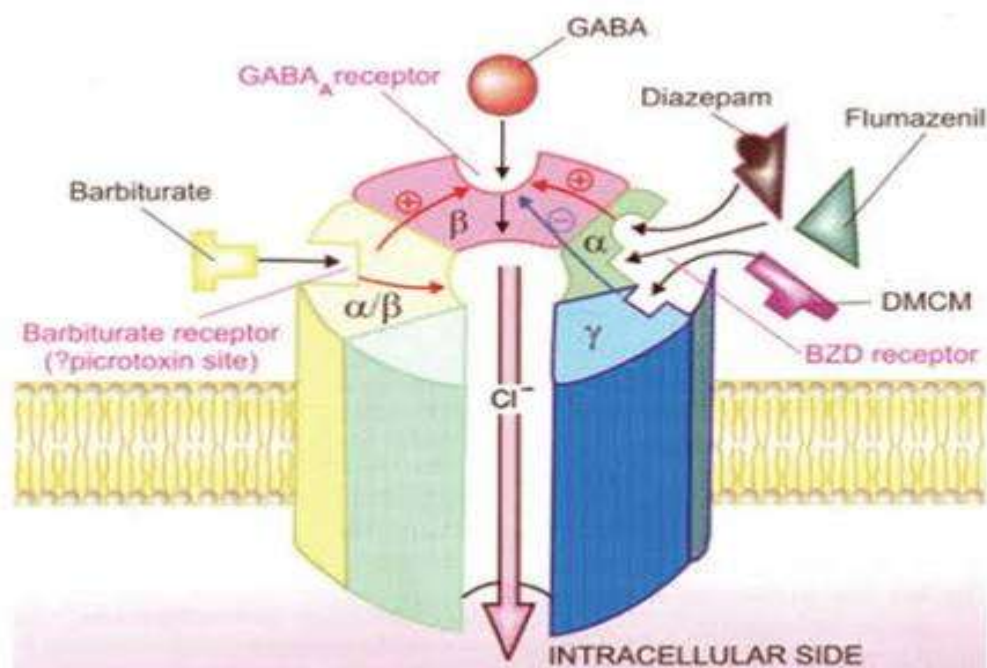


Fig - 7: Schematic depiction of GABA_A benzodiazepine receptor-chloride channel complex

Drugs affecting GABA_A receptor gated chloride channel

GABA	: Agonist at GABA _A site
Muscimol	: Endogenous agonist at GABA _A receptor → promotes Cl ⁻ influx
Bicuculline	: Competitive antagonist at GABA _A receptor
Picrotoxin	: Blocks o- channel noncompetitively; acts on picrotoxin sensitive site
Barbiturate	: Agonist at an allosteric site (? picrotoxin site); prolong GABA action; open o- channel
Alcohol, Inhalational anaesthetics, Propofol	: Open o- channel directly; allosteric facilitation of GABA
Benzodiazepine	: Agonist at an allosteric BZD site → facilitate GABA action
Beta –Carboline	: Inverse agonist at BZD site → impede GABA action (DMCM)
Flumazenil	: Competitive antagonist at BZD site ^[23]

Pharmacokinetics

Diazepam can be administered orally, intravenously (must be diluted, as it is painful and damaging to veins), intramuscularly (IM), or as a suppository.

When administered orally, it is rapidly absorbed and has a fast onset of action. The onset of action is one to five minutes for IV administration and 15–30 minutes for IM administration. The duration of diazepam's peak pharmacological effects is 15 minutes to one hour for both routes of administration. The bioavailability after oral administration is 100%, and 90% after rectal administration. Peak plasma levels occur between 30 and 90 minutes after oral administration and between 30 and 60 minutes after intramuscular administration; after rectal administration, peak plasma levels occur after 10 to 45 minutes. Diazepam is

highly protein-bound, with 96 to 99% of the absorbed drug being protein-bound. The distribution half-life of diazepam is two to 13 minutes.

When diazepam is administered IM, absorption is slow, erratic, and incomplete.

Diazepam is highly lipid-soluble, and is widely distributed throughout the body after administration. It easily crosses both the blood–brain barrier and the placenta, and is excreted into breast milk. After absorption, diazepam is redistributed into muscle and adipose tissue. Continual daily doses of diazepam quickly build to a high concentration in the body (mainly in adipose tissue), far in excess of the actual dose for any given day.

Diazepam is stored preferentially in some organs, including the heart. Absorption by any administered route and the risk of accumulation is significantly increased in the neonate, and withdrawal of diazepam during pregnancy and breast feeding is clinically justified. ^[24]

Medical uses

Anxiety disorders

Most commonly clinically significant are:

- 1. Traumatic stress**
- 2. Generalized anxiety disorders** –chronic state anxiety even without existing cause
- 3. Panic disorder** -patient avoids situations he may feel might go into panic
- 4. Social anxiety disorder** –patient avoids social interaction

Most commonly SSRI or SNRI and benzodiazepines are given. Benzodiazepines are usually the first line of treatment, as acute relief of symptoms occurs, but for longer term treatment tolerance and dependence occurs so SSRI and SNRI are drugs of choice.

5. Pain disorder

Ascending cortical pathways involving monoamines, responsible for some role in analgesia. Antidepressants have analgesic property in addition. Given in chronic pain associated with depression. Antidepressants are not given in normal routine as painful, non-responding debilitating pain known as neuropathic pain of DM, post hepatic neuralgic pain, trigeminal neuralgia, chronic backache. Most commonly TCAs and SNRI are used. Duloxetine is well known for this condition.

6. Premenstrual dysphoric disorders

Becomes irritable and change in mood occurs, which is usually mild. In some, troublesome signs, symptoms appear like fatigue, irritability, depression, seen in 2nd half of menstrual cycle (luteal phase), related somewhat to increased levels of progesterone.

SSRI – Fluoxetine has been used successfully, used either continuously or for these two weeks of luteal phase. Both ways equally effective.

7. Smoking cessation: Bupropion, has different mechanisms:

- Nicotine like action, norepinephrine, dopamine
- Antagonistic effect on nicotinic receptors.
- Nicotine has antidepressant property, substitute for nicotine. As effective as transdermal nicotine patch. Other drug is nortriptyline.

8. Miscellaneous

- Enuresis –children night bed wetting TCA are given, Imipramine, advantage is that also has anticholinergic property. Bladder control is improved. It has less sedating effects, child wakes up easily.

- For urinary stress and incontinence duloxetine is prescribed
- Vasomotor symptoms –premenopausal, menopausal if troublesome, antidepressants can be given SNRI Venlafaxine, nefazadone. As SSRI delay sexual functions, they are given in certain sexual dysfunctions like premature ejaculation. Chronic use depends on patient's profile, age, gender, and existing diseases. Also on drug tolerability and toxicity profile.

Specific clinical uses

SSRI	:	Depression, Anxiety disorder, Bulimia nevosa
5HT2 antagonist	:	Depression, Anxiety disorder, Insomnia
Tetracyclic and unicyclic antidepressants	:	Depression unresponsive, Bupropion –obesity
SNRI	:	Depression, Pain disorder, Anxiety disorder, Stress, Urinary incontinence,
TCAs	:	Depression unresponsive, Pain disorder, Enuresis, Insomnia
MAOs	:	Anxiety disorder, Selegiline –parkinson's disorder ^[20]

2. REVIEW OF LITERATURE

Phytochemical review of *Amaranthus viridis*.L

- **Sowjanya pulipati *et al.***, (2014) studied, Phytochemical and Pharmacological of *Amaranthus viridis*.L above presented information regarding *Amaranthus viridis* (L) is reviewed to congregate the ethno-botanical, phytochemical and pharmacological information. The plant was reported for various pharmacological activities, hence it has broad spectrum of activities in the treatment of numerous ailments. It was reported for the presence of few phytoconstituents responsible for few biological activities. Hence it is required to isolate the other phytoconstituents which can be used as lead molecules in synthesizing novel agents with good therapeutic activity. The isolation and characterization of phytoconstituents, elucidation of mechanism of action of isolated compounds and clinical trials of compounds are much needed. ^[25]
- **Savithramma N *et al.***, (2012) have reported Preliminary Phytochemical Analysis of Traditionally used medicinal Plants. The medicinal plants appear to rich in secondary metabolite, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, analgesic and diuretic can be attributed to their high phenols, tannins, triterpenoids, saponins, flavonoids. Exploitation of these pharmacological properties involves further investigation active ingredients by implementation of technique like extraction, purification, separation, crystallization and identification. ^[26]
- **Oliva c. Ruma.**, (2016) have been reported phytochemical screening of selected indigenous edible plants, In this study, although there are variations in the chemical constituents found to be present, the result gave comparative information about the main classes of secondary metabolites found in the indigenous edible plants evaluated. Therefore, the current findings revealed the richness of the indigenous

edible plants in different groups of active compounds that need to be studied for different applications may help achieve nutritional security.^[27]

- **Barnali Gogoi *et al.***, (2013) have been reported Phytochemical Constituents of Some Medicinal Plant Species Used in Recipe During ‘Bohag Bihu’ in Assam. An ethno botanical study has been carried out to focus on medicinal utility of 101 plant species eaten during Bohag Bihu in Assamese society. Amongst 101 species, 25 species are found to have effect on gastrointestinal problem, 18 species effect on skin diseases, 16 species effect on respiratory ailments, 14 species effect as anti diabetic, 7 species effect on gynaecological problems, 6 species effect as blood purifier, 4 species effect on rheumatism, 3 species effect on eye-sight improvement and 2 species effect on jaundice. These plants contain various phytochemicals like saponins, alkaloids, sterols, flavanoids, glycosides, terpenoids which have certain medicinal values. The paper reflects the rich ethno medicinal value of the herbs along with their phytochemical constituents The further scrutiny evaluation of the safety parameters of each component of the herb used in the recipe may be investigate to develop a pharmacologically potent lead molecule.^[28]
- **Sehrish sadia *et al.***, (2016) qualitative and quantitative phytochemical analysis and antioxidant potential of *Amaranthus viridis*.L by using different assays. The results of present study showed that extract of *A. viridis* leaves contain high amount of flavonoides and phenolic contents and exhibited high antioxidant activity. Previous documentations showed that high scavenging activity is related to the presence of hydroxyl group in phenolic compounds structure. As free radicals are highly involved in the pathogenesis of a lot of diseases so free radical scavengers can be a preventive measure for those diseases. Thus observed radical scavenging activity of *A. viridis*

leaf extract can be exploited for disease prevention as well as nutraceutical application.^[29]

- **Saud Asif Ahmed *et al.***, (2013) Phytochemical Profiling with Antioxidant and Antimicrobial Screening of *Amaranthus viridis* L. Leaf and Seed Extracts. From the study it was concluded that the edible plant species *Amaranthus viridis* from underutilized plant family had a rich amount of valuable ingredients that are beneficial for health^[30]
- **Liu D *et al.***, Studied the effects of different concentration (10(-6)M, 10(-5)M and 10(-4)M) of $K_2Cr_2O_7$ on some minerals (Mn, Fe, Cu and Zn), Lipid peroxidation, activities of antioxidants enzymes, Photosynthetic function, and chlorophyll fluorescence characteristics were investigated in hydroponically grown *Amaranthus viridis* L. Results indicated that chromium was accumulated primarily in roots^[31]
- **Larbie Christopher *et al.***, (2015) studied the effect of phytochemical and phenolic content, anti oxidant and anti proliferative effects of 50% ethanolic extract of *Amaranthus viridis* L. Leaves and stem using standard methods. *Amaranthus viridis* L. MTT assay, it was observed that all the extract had anti-proliferative activity against 3 leukemic cell lines (Jurkat, CEM and HL -60). The AVL had IC_{50} value of 111.41 μ g/ml (JURKAT), 122.5 μ g/ml (HL-60) and >1000 (CEM). It was also observed extract enhanced the proliferation of normal cells while the standard curcumin had an anti-proliferative effect on leukemic and normal cells. It can be concluded that the better anti oxidant and pro-liferative effect and could further be explored as a novel source of cancer therapy.^[32]

- **L. Prabhas et al.,** (2016) studied preliminary phytochemical analysis of some traditional medicinal plants, Qualitative phytochemical analysis of selected plant species confirms the presence of various phytochemicals like alkaloids, flavonoids, steroids and terpenoid. The presence of phytochemicals can be unified with medicinal potential of these plants. Research study reveals the scientific evidences associated with use of traditional medicinal plants for the treatment of various diseases among human beings.^[33]
- **Jana Kalinova et al.,** (2009) studies were conducted to confirm the presence of rutin, one of the most common quercetin glycosides, and other quercetin derivatives in plants of genus *Amaranthus*^[34]

Pharmacognostical review of *Amaranthus viridis*. L

- **Musharaf khan et al.,** (2010) studied the Pharmacognostic evaluation of the *Amaranthus viridis* L. (Family Amaranthaceae) plant was studied to determine the various parameters for pharmacognostical standards. The present investigation deals with the report on macro and microscopical, vein islet and vein termination numbers, palisade ratio, stomatal index (upper and lower surfaces of the leaf) and different chemical parameters have been determined. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.^[35]

Pharmacological review of *Amaranthus viridis*.L

- **B.S.Ashok Kumar et al.,** (2010) showed the invitro anthelmintic property of methanol extract of *Amaranthus viridis* Linn, Antinociceptive and antipyretic

Activities and (2009) estimation of Bioflavonoid in *Amaranthus viridis* Linn by HPLC^[36]

- **Girija K et al.,** (2011) to investigate the Methanol extract of *Amaranthus viridis*.L, *Amaranthus caudatus*.L, *Amaranthus spinosus*.L, showed significant anti-diabetic and anti-cholestrolemic activity, which proves the scientific proof for their traditional claims.^[37]
- **Vrushali jadhav et al.,** (2016) studied Evaluation of antioxidant activity of *Amaranthus viridis*.L Methanolic extract, in this study, we prepared and evaluated antioxidant activity of selected plant extracts. This result showed that tested plant products had noticeably antioxidant effect and total phenolic content. Antioxidant compounds from these products with preventive and treatment effect on various diseases can be used for improving the shelf life of food products. The present study may help in use of these naturally occurring species in the development of new drug^[38]
- **Kausar malik et al.,** (2016) have been reported the Anti bacterial activity of *Amaranthus viridis* L. In his study, Samples of medicinal plants were extracted using water and methanol and tested for their antimicrobial activities against pathogenic stains and usual strains of wound causing bacteria by disc diffusion method. Methanol extracts had comparatively more activities as compared to their corresponding aqueous extracts. Polarity of methanol and high stability of plant secondary metabolite in methanol could be probable reason for high extractive value. Chloroform extracts of *A. viridis* were found to show lesser inhibition than methanol and ethanol. This was supported by statistical analysis for both stem and leaves. This finding was reported by *Islam et al*^[39]

- **Sivaji Asha *et al.***, (2013) have been reported the Antiuro lithiatic Activity of *Amaranthus viridis*.L, In this studied ,the data revealed were suggested that *Amaranthus viridis*.L possess significant antiuro lithiatic activity^[40]
- **Ying-Shan Jin *et al.***, (2013) have been reported the Anticancer activities of extract from *Amaranthus viridis*.L, Study of demonstrated that ethyl ether fraction of *A.viridis*.L (EA) has an anti-proliferative effect by reducing cell viability, ROS generation, regulation of the caspase-3 gene, activation o Bax and Bcl-2 and cell cycle arrest. EA increases the level of ROS and apoptotic cell death in HT-29 cancer cells. Our results demonstrate that the cell apoptosis induced by EA induces G0/G1 cell cycle arrest and was associated with generation of ROS and enhanced expression of capase-3 and Bax/Bcl-2 ratio.^[41]
- **Ashok Kumar BS *et al.***, (2011) have been reported the Hepatoprotctive and antioxidant activities of *Amaranthus viridis*.L, In conclusion, administration of MeAv for liver protective activity against paracetamol induced liver damage the potential antioxidant property of MeAv thought to be the mechanism behind its hepatoproctive activity.^[42]
- **Adewale Adetutu *et al.***, (2016) studied in Inhibition of vivo growth plasmodium berghei by *launaea taraxacifolia* and *Amaranthus viridis*.L in mice.The results of this study can be used as basis for further phytochemical investigation in the search for new and locally affordable antimalarial agents.^[43]
- **Saikia *et al.***, (2015) study was to determine the anticonvulsants activity of methanolic extract of *Lawsonia inermis* leaves in albino rats. The anti convulsant activity of methanolic extract of leaves of *lawsonia inermis* (200mg/kg and 400mg/kg) was assessed in rats using maximum electroshock seizure (MES) test ant pentylenetetrazole(PTZ) induces seizure test. The study demonstrate that *Lawsonia*

inermis has significant anticonvulsant activity possibly through a GABA-ergic interaction.^[44]

- **O.O. Adeyemi *et al.***, (2010) studied investigate the anticonvulsant, anxiolytic and sedative activities of the aqueous root extract of *Securidaca longepedunculata*, These findings justify the use of *Securidaca longepedunculata* in traditional medicine for the management of convulsion and psychosis.^[45]
- **Jiban Debnath *et al.***, (2010) have been reported the anticonvulsant activity of ethanolic extract of *Terminalia chebula* in albino mice. Ethanolic extract of *Terminalia chebula* (EETC) possess anticonvulsant activity since it reduced the duration of seizures produced by maximal electroshock and delayed the latency of seizures produced by pentylenetetrazole and picrotoxin.^[46]
- **Nadithe Laxman Reddy *et al.***, (2016) have been reported demonstrated that *Citrus sinensis* possesses anticonvulsant activity against Maximal Electric Shock induced seizures in mice. The anticonvulsant activity was better with dose of 100mg/Kg as compared to 50mg/Kg. However its anticonvulsant properties were inferior to the standard drug Sodium valproate. It may only act as an adjuvant therapy along with standard anticonvulsant drugs in seizure prevention.^[47]
- **Saba hasan *et al.***, (2012) have been reported Anti epileptic activity of some medicinal plants. These remedies can make anti convulsant treatment more rationale and patient friendly. In conclusion, the medicinally important plant species, listed in the present paper appear to be promissory sources of anticonvulsant agents. The future outlook for the development of new antiepileptic drugs derived from these medicinal plants is therefore positive.^[48]
- **Jhansi Konduru *et al.***, (2014) studied, Even though the antidepressant drugs can treat the disorders like depression, but they have side effects too. Upon doctor's

suggestion only it is better to use within the limit and also use the appropriate drug in order to reduce the symptoms.^[49]

- **Ramiro Salas *et al.***, (2003) have reported Altered Anxiety-Related Responses in Mutant Mice Lacking the α_4 Subunit of the Nicotinic Receptor. we showed that the lack of the nAChR α_4 subunit alters the behavioral responses to certain anxiety-provoking experimental paradigms. The effect might be dependent on the MHb–IPN expression of the α_4 subunit. The data on α_4 , α_3 , and α_4 co expression might help to explain how different nAChR types can exert their influence on anxiety-related behavioral tests.^[50]
- **Angelika Roedel *et al.***, studied Effects on light or dark phase testing on behavioural and cognitive performance in DBA mice. From the results we conclude that testing during the light phase induces a pronounced behavioural inhibition as well as a cognitive disruption in DBA mice, which should be taken into account when cognitively testing these animals.^[51]
- **John Michael Holden *et al.***, (2015) have been reported Behavioral effects of mefloquine in tail suspension and light/dark tests. study concludes that acute administration of mefloquine leads to some behaviors indicative of emotional disinhibition in mice, including increased rearing and time in light in the light/dark apparatus and reduced immobility in the tail suspension test.^[52]
- **Michel Bourin *et al.***, (2003) have been reported to be an index of activity-exploration because of habituation over time, and the time spent in each compartment to be a reflection of aversion. Classic anxiolytics (benzodiazepines) as well as the newer anxiolytic-like compounds (e.g. serotonergic drugs or drugs acting on neuropeptide receptors) can be detected using this paradigm. It has the

advantages of being quick and easy to use, without requiring the prior training of animals.^[53]

- **Vinod H. Gupta *et al.***, (2012) have been reported Neuropharmacological Evaluation of the Methanolic Extract of *Couroupita guianensis* Aubl. Flower in Mice. Appears that the methanolic extract of *C. guianensis* can cause a central nervous system depression indicates active sedative properties which may be the basis of its folkloric use in traditional medicine to reduce pain. However our results need to be further corroborated with different models to speculate on the mechanism of action.^[54]
- **Digambar B. Ambika *et al.***, (2014) have been reported Evaluation of neuropharmacological activity of petroleum ether, Methanolic and aqueous extracts of flower heads of *Sphaeranthus Indicus* in mice SIP and SIM demonstrated significant anxiolytic and anticonvulsant activity. SIM and SIA showed improvement in discrimination index. More over SIM showed anti-hypoxic activity and delayed sodium nitrate induced respiratory arrest.^[55]
- **Moli Akter *et al.***, (2011) have been reported Evaluation of analgesic, neuropharmacological and cytotoxic activity of *Trigonella foenum-graecum* Linn. Phytochemical screening of the extract showed that the *Trigonella foenum-graecum* possess fibers, flavonoids, polysaccharides, saponins, flavonoids and polysaccharides fixed oils and some identified alkaloids viz., trigonelline and choline. The analgesic, CNS depressant and cytotoxic properties of *Trigonella foenum-graecum* observed in animal model might, in part, be due to the presence of such compounds. The results also suggest a rationale for the traditional uses of this plant. However, studies are required on higher animal model and subsequently on

human subjects to prove its clinical efficacy as an analgesic, CNS depressant and cytotoxic agent.^[56]

- **Mohammad Shahriar et al.,** (2014) have been reported analgesic and neuropharmacological activity of *withania somnifera* root, After pharmacological studies with root extracts of *Withania somnifera* for analgesic activity and neuropharmacological investigation, plant root has significant analgesic activity and neuropharmacological action.^[57]
- **Yogesh Chand Yadav et al.,** (2013) have been reported Neuropharmacological screening techniques for pharmaceuticals. This screening model can be use to study of memory enhancing, Anxiolytic, Antidepressant and anticonvulsant activity^[58]

Ethno botanical Information of *Amaranthus viridis*.L

- Leaf sap is used as an eye wash treat eye infections, convulsions and epilepsy in children: powder leaf contains reducing sugars and resins that have allolipathic effects on lettuce seed germination, A Review of Nutritional value and utilization of *Amaranthus* (AMARANTHUS SPP).^[59]
- Leaves is orally administered for fistula and piles, ethnomedical survey was carried out in the five villages of Narsinghdi district, Bangladesh.^[60]
- Leaves are having the Antimicrobial, antioxidant, laxative, anti-inflammatory, skin infection etc. (2016) 2nd international conference on recent innovation technology, management and enviorement.^[61]

3. PLANT PROFILE



Fig-8 :Plant of *Amaranthus viridis* Linn

GENERAL INFORMATION:

Common name	- Green Amaranth, pigweed, Prince of Wales feather, slender amaranth, Tropical green amaranth
Synonym	- <i>Amaranthus gracilis</i> , <i>Amaranthus polystachyus</i> , <i>Euxolus viridis</i>
Family	- Amaranthaceae

TAXONOMICAL CLASSIFICATION:

Kingdom	- Plantae - plants
Subkingdom	- Tracheobionta – Vascular plants
Family	- Amaranthaceae
Superdivision	- Spermatophyta – seed plants
Division	- Magnoliophyta – Flowering plants
Class	- Magnoliophyta – Dicotyledons
Subclass	- Caryophyllidae
Order	- Caryophyllales
Family	- Amaranthaceae – Amaranth family
Genus	- <i>Amaranthus</i> L. – pigweed
Species	<i>Amaranthus polygonoides</i> L. – Tropical amaranth
-	

VERNACULAR NAME:

Tamil	- Kuppaikeerai
Malayalam	- kuppacheera
Hindi	- Jungali chaulayl
Telugu	- Chilaka – thotakoora
Marathi	- Unadabhaji
Sanskrit	- Tandulya ^[62]

BOTANICAL DESCRIPTION

A. viridis is an annual herb with an upright, light green stem that grows to about 60-80 cm in height. Numerous branches emerge from the base, and the leaves are ovate, 3-6 cm long and 2-4 cm wide, long petioles of about 5 cm. The plant has terminal panicles with few branches, and small green flowers.

ECOLOGY

It requires well drained fertile soil in a sunny position. It should not be provided with inorganic fertilizer. It is cultivated as a food in tropical countries. It photosynthesis by C₄ carbon-fixation pathway which effects at high temperature.

PROPAGATION

A. viridis is propagated by sowing seeds in spring. Germination is rapid if the soil is warm. A drop in temperature overnight will assist the germination. Apart from seeds the cuttings of growing plants are also used for propagation which roots easily.

USES

- The herb is used as astringent, emollient, in dysentery, inflammation, constipation, eczema, bronchitis, antidiabetic, anaemia and leprosy, Plant is used as sag for cooking and fodder plant.
- Leaves are emollient and anthelmintic. Roots/ shoots are used to control excessive menstruation, blood purifier, digesting agent, piles.
- The Negros of the Philippines apply the bruised leaves directly to eczema, psoriasis, and rashes with good results.
- The leaves make a good emollient preparation available in some of the Filipino villages for insect bites, sunburn, and regular burns.
- The reddish-brown fiber from the leaves are soaked and use for eye treatments.
- The decoction of young roots is used for the treatment of respiratory complaints, asthma.
- Provides energy: Highly packed with carbohydrates, proteins, vitamin K, folate, riboflavin, vitamin A, vitaminB6, and vitamin C, amaranth leaves boost energy in the body.
- Prevents electrolyte imbalance: Amaranth leaves are terrific source of manganese, iron, copper, calcium, magnesium, potassium and phosphorus necessary for maintaining proper mineral balance in the body.
- Improves digestion: High dietary fiber content (3 times that of wheat) in the greens improve digestive health and reduces constipation. It is easily digestible and good for both young ones and elders.
- Aids in weight management: Protein in the leaves help to reduce insulin levels in the blood and also release a hormone that lessen hunger pranks and prevent "binging catastrophe".

- Reduces bad cholesterol: One of the key benefits of amaranth leaves is cholesterol-lowering ability. Being fibrous, this leafy vegetable is effective in reducing LDL levels in the blood and promotes weight loss. Presence of tocotrienols (a type of vitamin E) also aids in cholesterol-lowering activity.
- Good for anemic patients: Iron-rich (5 times that of wheat) red amaranth leaves promote coagulation and increase hemoglobin content and red blood cell counts.
- Decreases risk of cardiovascular disease: Amaranth leaves are an excellent dietary source of phytosterols that lowers blood pressure and prevents heart ailments including stroke.
- Fight-off cancer: Presence of lysine (an essential amino acid) along with vitamin E, iron, magnesium, phosphorus and potassium and vitamin C helps to fight against free radicals responsible for aging and formation of malignant cells.
- Ayurvedic treatments: Juice extracted from fresh amaranth leaves are prescribed for treating diarrhea, and hemorrhage conditions.
- Stop hair loss and graying: Besides regular consumption, applying juice from the leaves prevent brittle hair falling. This wonderful cosmetic benefit of amaranth leaves also retards the onset of premature graying.
- Prevents calcium-deficiency ailments: Calcium present in amaranth leaves reduce risk of osteoporosis and other calcium deficiencies because it has twice the calcium as milk. Indeed good news for lactose-intolerance.
- Improves eyesight: Vitamin C found in the leaves contribute to towards healthy vision.
- Green amaranth also has clusters of nutty edible seeds, which can be eaten as snacks or used in biscuits. A porridge can be made by boiling the seeds in water. Unlike other amaranths, the seeds can be easily harvested by scraping the ripe spikes of seeds between the fingers. ^{[63]H6}

4. AIM AND OBJECTIVE

AIM

Now-a-days, there is an increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to allopathic drugs. Literature survey has revealed that the plant *Amaranthus viridis*.L is prescribed in the treatment of diabetes, anti-cholesterolemic, anti-pyretic and anti-nociceptive, hepatoprotective, anti oxidant conditions etc The plant, *Amaranthus viridis*.L was selected for the present study its,

- a) Easy availability
- b) Good therapeutic activity
- c) Degree of research work which is not done

Very less pharmacological studies have been carried out on the plant *Amaranthus viridis*.L. As per the literature review, so far no scientific study has been carried on this plant *Amaranthus viridis*.L (Family: *Amaranthaceae*) to explore the neuroprotective activity. So, the present study has been under taken to explore the neuropharmacological effect of leaves of methanolic extract of leaves of *Amaranthus viridis*.L which includes studies on pharmacological, Preliminary phytochemical and its pharmacological evaluation.

OBJECTIVES

- a) To determine the phytochemical constituents present in the methanolic extract of leaves of *A.viridis* L.,
- b) To investigate methanol extracts of leaves of *Amaranthus viridis* .L (*Amaranthaceae*) for various neuropharmacological activities, such as anti convulsant, anti depressant and anxiolytic

5. PLAN OF WORK

The present investigation was focused on the following:

- Collection of plant
- Authentication of the plant
- Extraction of plant material with methanol
- Pharmacognostical studies
 - A. Transverse section of leaves
 - B. Analytical Parameters
 - a) Ash value
 - b) Extractive value
 - c) Loss on drying
- To carry out the preliminary phytochemical screening of the extract
- To carry out the pharmacological screening of the methanolic extract of Leaves of *Amaranthus viridis*.L for,
 - ❖ **ANTI – CONVULSANT ACTIVITY**
 - a) Maximal electroshock –induced convulsion (MES)
 - ❖ **ANTI – DEPRESSANT ACTIVITY**
 - a) Forced swimming test (FST)
 - b) Tail suspension test (TST)
 - ❖ **ANTI - ANXIETY ACTIVITY**
 - a) Elevated plus maze test (EPM)
 - b) Light- dark Test (LDT)
- Statistical Analysis
- Documentation of results

6. MATERIALS AND METHODS

Collection, identification and authentication of plant material:

The plant of *Amaranthus viridis*.Linn was collected in the month of January, 2017 from the house garden, Nasiyanur, Erode district, Tamil nadu, India. The plant material was identified and authenticated by Dr. A. Balasubramaniam, Research Consultant, ABS Botanical garden, Kaaripatti, Salem District, Tamil nadu.

Shade drying, Granulation and Extraction:

The plant leaf were taken and dried in shade. Then the shade dried leaf were coarsely powdered by means mix grinder and was through sieve no 60 to get the coarse powder. Then the coarsely powdered materials were weighed, packed in an airtight container and used for extraction with solvent, phytochemical studies and pharmacological studies.

METHOD OF EXTRACTION

Soxhlet extraction:

Soxhlet extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor for several times. This process involves in the extraction followed by evaporation of the solvent. The vapours of the solvent are taken to a condenser and the condensed liquid is returned to the drug for continuous extraction. Soxhlet apparatus designed for such continuous extraction consists of a body of extractor attached with a side tube and siphon tube. The lower of the extractor is attached to distillation flask and the mouth of extractor is fixed to a condenser by the standard joints. The powdered crude drug is packed in the soxhlet apparatus directly or in a thimble of filter paper or fine muslin cloth. The diameter of the thimble corresponds to the internal diameter of the soxhlet extractor. Extraction assembly is set up by fixing condenser and a distillation flask. Initially

for the setting of the powder, solvent is allowed to once before heating. Fresh activated porcelain pieces are added to the flask to avoid bumping of the solvent. The vapours pass through the side tube and the condensed liquid gradually increase the level of liquid in the extractor and in the siphon tube. A siphon is set up as the liquid reaches the point of return and the contents of the extraction chamber are transferred to the flask. The cycle of solvent evaporation and siphoning back can be continued as, many times as possible changing the solvent, so as to get efficient extraction. This method although a continuous extraction process, is nothing but a series of short macerations. ^[64]

Preparation of plant extract:

About 350 gm of coarsely powdered leaf was packed in 1000ml soxhlet apparatus and extract with methanol for 72 hours by continuous hot percolation. After extraction, the solvent was distilled off and extract was concentrated to at room temperature and the percentage yield was calculated.

PHARMACOGNOSTICAL STUDIES**Tranferse section of leaves of (T.S) *Amaranthus viridis*.L**

T.S of petiole, T.S of midrib, T.S of lamina, venation pattern was done and its procedure was given below as follow.

Fixation

Care was undertaken to select healthy plant and normal organs. The required sample of different was cut and removes from the plant and fixed in FAA (Formalin -5ml+ acetic acid 5ml+70% ethyl alcohol 90ml). After 24 hour of fixing the specimen were dehydrated with graded series of tertiary butyl alcohol. ^[65] Infiltration of specimen was carried out by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning:

The paraffin embedded specimens were sectioned with the hand and dewaxing of section was customary procedure. ^[66] The sections were stained with toluidine blue. Since toluidine blue is polychromatic stain. The staining results were remarkable good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose wall, blue to the lignified cells, dark green to suberin, violet to mucilage, blue to protein bodies etc. Where ever necessary sectioned were also stain with safranin and IKI (for starch) ^[67]

For studying the stomatal morphology, venation pattern and trichome distribution, Para dermal section (section taken parallel to the surface of leaf) as well as clearing of leaf with the 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffery's maceration fluid were prepared. Glycerin mounted temporary preparation were made for macerated /cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrograph:

Microscopic descriptions of tissues are supplement with micrographs where ever necessary. Photograph of different magnification were taken in Nikon labphoto 2 microscope units. For normal observations bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under the polarized light they appear bright against dark back ground. Magnifications of the figure are indicated by the scale bars ^[68].

ANALYTICAL PARAMETERS

Ash values, extractive value, loss on drying were used for the study of physical properties.

Ash value

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is therefore, an important parameter for purpose of evaluation of crude drugs. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small and any marked difference indicates a change in quality. The ash value can be determined three different methods to measure the total ash, the acid insoluble ash and water soluble ash.

Determination of total ash value

Weigh accurately about 5gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon cooled weighed then calculated the percentage of total ash with reference to air dried drug.

Determination in of acid insoluble ash value

Boil the total ash with 25ml of 2M Hcl for 5 minute, collect the insoluble matter in a gooch crucible or on an ash less filter paper and wash hot water, ignite then cool in a desiccators and weight. Calculate the percentage of acid insoluble ash with reference to the air dried drug.

Determination of water soluble ash value

Water soluble ash is that part of the total ash content which is soluble in water. It is good indicator of either previous extraction of the water soluble salts in the drugs of incorrect preparation.

To the crucible containing the total ash, add 25ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered glass crucible or on an ashless filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450° c. Subtract the weight residue in mg from the weight of total ash. Calculate the content of water soluble ash in mg/g of the air dried material.

Determination of sulphated ash value

Heat silica of platinum crucible to redness for 10 minutes; allow cooling in desiccators and weighing. Unless otherwise specified in the individual monograph, transfer to the crucible 1 gm of the substance under examination and weigh the crucible and the contents accurately. Ignite, gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of H₂SO₄ heat gently until the white fumes are no longer evolved and ignite at 800°± 25°C until all black particles have disappeared. Conducted the ignition in a place protected from air current.

EXTRACTIVE VALUES

This method is to determine the amount of active constituents in a given amount of medicinal plant material when extracted with solvent. It is employed for that material for which no chemical or biological assay.

Determination of alcohol soluble extractive value

5gm of air dried and coarsely powdered drug has to be macerated with 100ml of ethanol of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, filter rapidly taking precautions

against loss of ethanol. Evaporate 25 ml of filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105°C and weigh. The percentage of ethanol soluble extractive value with reference to the air dried drug has to be calculated.

Determination of water soluble extractive value

The water soluble extractive value plays an important role for the evaluation of crude drugs. 5gm of the air dried and coarsely powdered drug as to be macerated with 100 ml of water of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing for 18 hours. Thereafter, filter rapidly taking precautions against loss of water. Evaporate 25 ml of filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105°C and weigh. The percentage of water soluble extractive value with reference to the air dried drug has to be calculated.

LOSS ON DRYING

Loss on drying is the loss of mass expressed as percentage w/w. It determines both water and volatile matter in the crude drug. It can be carried out either by heating at 100-105°C or in desiccators over phosphorus pent oxide under atmospheric or reduced pressure at room temperature for specified period of time.

Procedure

About 2 gm of powdered drug was taken in a tarred porcelain dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. Percentage of loss drying with reference to the air dried substance was calculated.^{[69][70]}

PRELIMINARY PHYTOCHEMICAL ANALYSIS

1. TEST FOR CARBOHYDRATES

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the absence of carbohydrates and glycosides.

a) Molich's test:

The filtrate was treated with 2-3 drops of 1% alcoholic α -naphthol solution and 2 ml of concentrated sulphuric acid was added along the test tube. Dis-Appearence of brown ring at the junction of two liquids shows presence of carbohydrates.

b) Fehling's test:

The filtrate was treated with 1 ml of Fehling's solution A and B and heated on the water bath. A reddish precipitate was obtained shows the carbohydrate.

c) Test for pentose sugar:

Mix equal amount of test solution and HCL. Heated and added the crystal of phloroglucinol. Presence of carbohydrates.

2. TEST FOR FIXED OIL AND FATS

a) Spot test:

Small quantity of the extract was pressed two filter paper, No appearance of oil stain on the paper indicates the absence of fixed oil.

b) Saponification Test:

Add a few drops of 0.5N alcoholic potassium hydroxide to a small quantity of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1-2 hrs. No changes occur indicates the absence of oil and fats.

c) Treat 5 drops of sample with 1% sulphate solution, and then add 10% sodium hydroxide solution. A clear blue solution is obtained which shows glycerine is present

in the sample. The cupric hydroxide formed in the reaction does not precipitated out as it solution in glycerine.

3. TEST OF PROTEINS

a) Biuret Test:

To the extract solution (2 ml) and biuret reagents was added, Violet colour indicates the presence of protein.

b) Xanthoprotein Test:

To 5 ml of extract solution, 1 ml of nitric acid was boiled, yellow precipitate was formed. After cooling it, add 40% sodium hydroxide solution orange colour was formed.

c) Millions Test:

2 ml of the test solution was mixed with millions reagent, white precipitate was formed.

4. TEST FOR STROIDS

a) Salkowski's Test:

To 1 ml of chloroform solution, few drops of concentrated sulphuric acid were added. Brown colour produced showing the presence phytostrols.

b) Libermann – berchard's Test:

The extract was added with few drops of acetic anhydride. Boiled and cooled. Then concentrated sulphuric acid was added from the side of the test tube, brown ring was formed at the junction of two layers and upper layer turned green which showed presence of steroids.

5. TEST FOR GLYCODIDES

TEST

a) Test A:

200 mg of the drug was extracted with 5ml of dilute H_2SO_4 by warming on a water bath. It was filtered and then the acid extracted was neutralized with 5% solution of sodium hydroxide. 0.1 ml of fehling's solution A and B added until it became alkaline and heated on a water bath for 2 minutes, the quantity of red precipitate formed was noted and compared with that of formed in test B.

b) Test B:

200gm of drug was extracted with 5 ml of water instead of H_2SO_4 . After boiled equal amount of water was added. 0.1 ml of fehling's solution A and B was added until it becomes alkaline and heated on a water bath for 2 minutes. The quantity of red precipitate formed was noted. The quantity of precipitate formed in test B with that formed in test A was compared. And it shows the presence of glycosides.

6. TEST FOR CARDIAC GLYCOSIDE

a) Baljiet's Test:

The extract was mixed with picric acid or sodium picrate. Orange colour was formed. Indicates the presence of glycosides.

b) Legal's Test:

To the alcoholic solution of extract, 1 ml pyridine and 1 ml sodium nitro-pruside solution, blood red colour was observed shows the presence of cardiac glycosides.

c) Keller-killiani Test:

To 2 ml of the extract, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride were added. This solution was carefully transferred to the surface of 2ml concentrated H_2SO_4 and the observed was noted down.

7. TEST FOR ANTHRAQUINONE GLYCOSIDES

a) Borntrager Test:

The test material was boiled with 1 ml H_2SO_4 in a test tube for five minutes. It was filtered while hot, the filtrate was cooled and shaken with equal volume of dichloromethane or chloroform. The lower layer of dichloromethane or chloroform was separated and shaken it with half of its volume of dilute ammonia. No rose pink to red colour was produced in the ammonical layer.

b) Modified Borntrager Test:

200 mg of the material was boiled with 2 ml of dilute H_2SO_4 . It was treated with 2 ml of 5% aqueous ferric chloride solution for 5 minutes. It was shaken with equal volume of chloroform. The organic solvent layer were separated and add equal volume of dilute ammonia was added, ammonia layer shows pinkish red colour. Shows the presence of glycosides.

8. TEST FOR FLAVONOIDS

a) Shinoda's Test:

To the extract solution few magnesium turning were added and concentrated HCl was added drop wise, pink scarlet, red appeared after few minutes showed its presence of flavonoids.

b) To small quantity of residue lead acetate solution was added & the colour changes was observed.

9. TEST FOR FATS AND OILS

a) Solubility Test:

i) To 2-3ml of the extract, few ml of chloroform was added and solubility was observed.

- ii) To 2-3ml of the extract few ml of 90% methanol was added and solubility was observed

10. TEST FOR TANNINS AND PHENOLIC COMPOUNDS

a) Ferric chloride Test:

To the test solution, ferric chloride solution was added, green colour appeared showing the presence of condensed tannins.

b) Phenazone test

To the test solution, 0.5 grams of sodium phosphate was added, warmed and filtered. To the filtrate 2% phenazone solution was added, bulky precipitate was formed which was often coloured, indicating the presence of tannins.

11. TEST FOR ALKALOIDS

The extract is evaporated separately. To the residue dilute HCL was added and it was shaken well and filtered. The following tests were performed.

a) Dragendroff's Reagents:

To 2-3ml of filtrate few drops of dragendroff's reagents were added and precipitate was observed. It indicates the presence the alkaloid.

b) Mayer's Test:

To 2-3ml of filtrate few drops of mayer's reagents were added and precipitate was observed. It indicates the presence of alkaloids.

c) Hager's Test:

To 2-3 ml of filtrate few drops of hager's reagents (saturated solution of picric acid) were added and precipitate was observed. It indicates the presence of alkaloids.

d) Wagner's Test:

To 2-3 ml of filtrate few drops of wagner's reagents were added and the precipitate was observed. It indicates the presence.

12. TEST FOR AMINO ACIDS:**a) Ninhydrin Test:**

3ml of the test solution was heated with 3 drops of 5% ninhydrin solution in a boiling water bath for 10 minutes and then the dark blue colour was observed. Shows the presence of amino acids.

b) Millon's Test:

To test solution about 2ml of millon's reagents was added. A white precipitate was obtained indicating the presence of amino acids. ^{[71][72]}

PHARMACOLOGICAL STUDIES

ANIMAL STUDIES

Source and Maintenance of Experimental Animals

Healthy male and female adult rat & mice, weighing between 20-25 g & 150-250 g were obtained from the Animal House of the KMCH College of pharmacy, Coimbatore. The animals were housed under standard environmental conditions, with feed and water provided *ad libitum*. Proper handling and using of the animals were in accordance with the guidelines and regulations, monitored and approved by the Ethical Committee on Animal use, Department of Pharmacology, KMCH College of pharmacy, Coimbatore

ANIMAL MODEL OF CONVULSION

Maximal Electro Shock (MES) induced Convulsion

Principle

The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally active drugs.

Procedure

The maximum electrical shock (MES) induced convulsion in animals represented grand mal type of epilepsy. These are type of procedures use to studies convulsions and to test to anticonvulsant drugs in laboratory animals. In MES convulsions electric shock is applied through the corneal electrode, through optic stimulation cortical excitation are produced .The MES –convulsion are divided into five phase such as Tonic flexion, Tonic extensor, Clonic convulsions, stupor, recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolished the extensor phase of MES convulsions. This procedure may be used to produce convulsions in rat . In this method place corneal electrodes on the cornea and apply the prescribed current and different stages of conclusions

are noted as in previous paragraph. Note the time (sec) spent by the animal in each phase of the conclusions. Inject phenytoin (i.p) in rats. Wait for 30 min and subject the animals to electro-convulsions as described. Note the reduction in time or abolition of tonic extensor phase of MES convulsions.^[73]

Experimental Design

Wistar albino rats weighed around 150-250g were used for the study. Rats were divided into four groups of 5 animals each,

- Group-1 : Received, normal saline (0.1% solution)
- Group-2 : Received standard drug, Phenytoin (20mg/kg *i.p.*)
- Group-3 : Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose)
- Group-4 : Received methanol extract of leaves of AV (500mg/kg; *p.o.* High dose)

ANIMAL MODELS OF ANTI-DEPRESSANT

Antidepressant activity was indicated the mood elevating due to various mechanism of the antidepressant drugs, such as inhibition of the enzyme of monoamine oxidase, inhibition of reuptake bioamines and enhancement of the concentration of 5-HT e) ct .Later on, inhibition of reuptake of bioamines was found to be main mechanism of action to downregulation of β receptor. Several lines of preclinical and clinical evidence indicates that enhancement of 5-HT mediated neurotransmission might underline the therapeutic effect of most of the antidepressant This behavioural effect very similar to that found by other author after treating mice with classical antidepressant drugs as IMI^[74] .various models are like,

Forced swimming Test (FST)

Principle

Behavioural despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state

of despair which can reduce by several agents which are therapeutically effective in human depression.

Procedure

The procedures for Forced swim test or Despair swim test were similar to those first described by **Porsolt, *et al.*** (1977). Male albino mice weighing 20-22 g are used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon® cages with free access to food and water. This test is sensitive to all major classes of antidepressant drugs, hence used as an animal model of depression like behaviour. Mice were grouped into three of five animals each and were trained individually for three consecutive days (pre-test session). In the “test-session” The animals were made to swim individually in an open cylindrical container of diameter 20cm and height 50cm, containing 25cm of water at 25°C, for a test period of 6 minutes. After a brief period of vigorous activity for about two minutes, mice maintain a typical immobile posture. The animals were considered immobile when they float in an upright position, making only negligible movements to maintain their head above water. The total duration of immobility was recorded and changes in the same were studied for various treatment groups. ^[75]

Experimental Design:

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each ,

Group-1 : Received normal saline (0.1% solution)

Group-2 : Received standard drug, imipramine (60mg/kg.*i.p*)

Group-3 : Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose)

Group-4 : Received methanol extract of leaves of AV (500mg/kg; *p.o.* High dose)

Tail suspension Test (TST)

Principle

The tail suspension test (TST) was developed as a rodent screening test for potential (human) antidepressants drugs. It is based on the assumption that an animal will actively try to escape an aversive (stressful) stimulus. If escape is impossible, the animal will eventually stop trying (“give up”). In the TST a mouse is suspended by the tail so that its body dangles in the air, facing downward. The test lasts for ten minutes and may be repeated multiple times. Mice initially struggling and hangs immobile it is considered to have “given up”. Longer periods of immobility are characteristic of a depressive-like state. The validity of this test stems from the finding that treatment with an anti-depressants drug will decrease the time the animal spends immobile.

Procedure

The total duration of immobility induced by tail suspension was measured according to the method described by *Steru et al* (1985). Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by an adhesive tape placed approx. 1cm from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.^[76]

Experimental Design

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each ,

Group-1 : Received normal saline (0.1% solution)

Group-2 : Received standard drug, imipramine (60mg/kg.i.p)

Group-3 : Received methanol extract of leaves of AV (250mg/kg; p.o. Low dose)

Group-4 : Received methanol extract of leaves of AV (500mg/kg; *p.o.* High dose)

ANIMAL MODELS FOR ANTI-ANXIETY

The animal model is considered one of most widely validated tests of assaying sedative and anxiolytic substances such as benzodiazepines. The test drug induced anxiolytic effect beginning at lower doses employed. An increase of most important variables of EPM test was found as follow; the percentage of time of mice spend on the open arms as well as the percentage of entries in the dark arms. The anxiolytic effect is also evidenced through light and dark test. As with the EPM test, this model is useful for modeling of anxiety. The low dose dependent effect could be attributed to biological variability, as well as chemical complexity of the test drug. Various model of anti anxiolytic testing are,

Elevated Plus-Maze Test (EPM)

Principle

Out of many possibilities to modify maze tests e.g. water maze, the Y-maze, the radial maze, and the elevated plus maze have found acceptance in many laboratories. The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.

Procedure

The plus maze for mice (Lister, 1987) consisted of two perpendicular open arms (30×5cm) and two closed arms (30×5×15cm) also in perpendicular position. The open and closed arms were connected with each other by a central platform (5×5cm). The lateral walls of the closed arm and floor of each arm were made of wood. The platform and lateral walls were painted in black. The maze was elevated at a height of 45cm above the floor. The animals were trained for two consecutive days (pre-test session). In the test-session, 30 minutes after the treatment with standard and sample, the animal was placed at the center of

the maze with its nose facing in the direction of one of the closed arms. The duration of the test period was 10 minutes, during which the following parameters were evaluated.

- The number of entries into the open arm.
- The number of entries into the closed arm.
- Time spent in the open arm.
- Time spent in the closed arm.

The experiment was carried out in a sound attenuated room. An entry was counted when the animal placed all its four paws on an arm.^[77]

Experimental Design

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each ,

Group-1 : Received normal saline (0.1% solution)

Group-2 : Received standard drug, diazepam (4mg/kg.*i.p*)

Group-3 : Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose)

Group-4 : Received methanol extract of leaves of AV (500mg/kg; *p.o.* High dose)

Light-Dark model

Principle

The light compartment is believed to be more aversive to the mouse than the dark compartment. Anxiolytic drug administration increases the number of entries to the light compartment; time spent in the light compartment and reduces freezing behaviour.

Procedure

The apparatus consists of a Plexiglas box with two compartments (20 x 20 cm) one of which is illuminated with a white light while the other remained dark. Each animal is placed at the centred of the illuminated compartments; facing one of the dark places, as well as the number of entries in each space is recorded for 3 min.^[78]

Experimental Design

Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into five groups of 5 animals each ,

Group-1 : Received normal saline (0.1%)

Group-2 : Received standard drug, imipramine (60mg/kg.i.p)

Group-3 : Received methanol extract of leaves of AV (250mg/kg; *p.o.* Low dose)

Group-4 : Received methanol extract of leaves of AV (500mg/kg; *p.o.* High dose)

Statistical Analysis

All the data were expressed as the mean \pm standard error of the mean (SEM). The statistical significance of the difference between the groups was analyzed by using Graphpad 5.0 software (Graphpad, San Diego, USA) by applying one way analysis of variance (ANOVA) followed by Dunnett's test as post hoc and also student's paired T-test. The value of $P < 0.05$ was considered to be statistically significant.

7. RESULT AND DISCUSSION

Table -4: Data showing colour, Consistency and yields of methanolic extract of powdered leaf of *Amaranthus viridis* Linn

S.NO	EXTRACT	COLOUR	CONSISTENCY	%YIELD (W/W)
1.	Methanolic Extract	Dark Green	Sticky Mass	12 % (w/w)

PHARMACOGNOSTICAL STUDIES

Transverse section (T.S) of leaves of *Amaranthus viridis*.L**T.S of petiole of leaves *A.viridis*.L**

Fig: 9. T.S of petiole of leaves of *Amaranthus viridis*.L

(Tr: Trichome, Ep: Epidermis, Pa: Parenchyma, Vb: Vascular bundle)

Description**Epidermis:**

The outer most layer of the petiole is epidermis. This is a single layer of parenchymal cells with anticlinal cell wall, almost circular, covered by cuticle. The stomata and trichomes are very rarely found. Tannins are found as a cell inclusion.

Cortex:

Cortex is made up of polygonal, collenchymatous parenchyma, compactly packed, no intercellular spaces, heavy in cellulose, thick walled.

Vascular bundle:

Numerous vascular strands are seen in the middle portion of petiole. They are bicollateral covered by pericyclic fibre which made up of lignified sclerenchyma cells. Xylem appears as round cells with high lignin content. Phloem is non lignified surrounding the xylem.

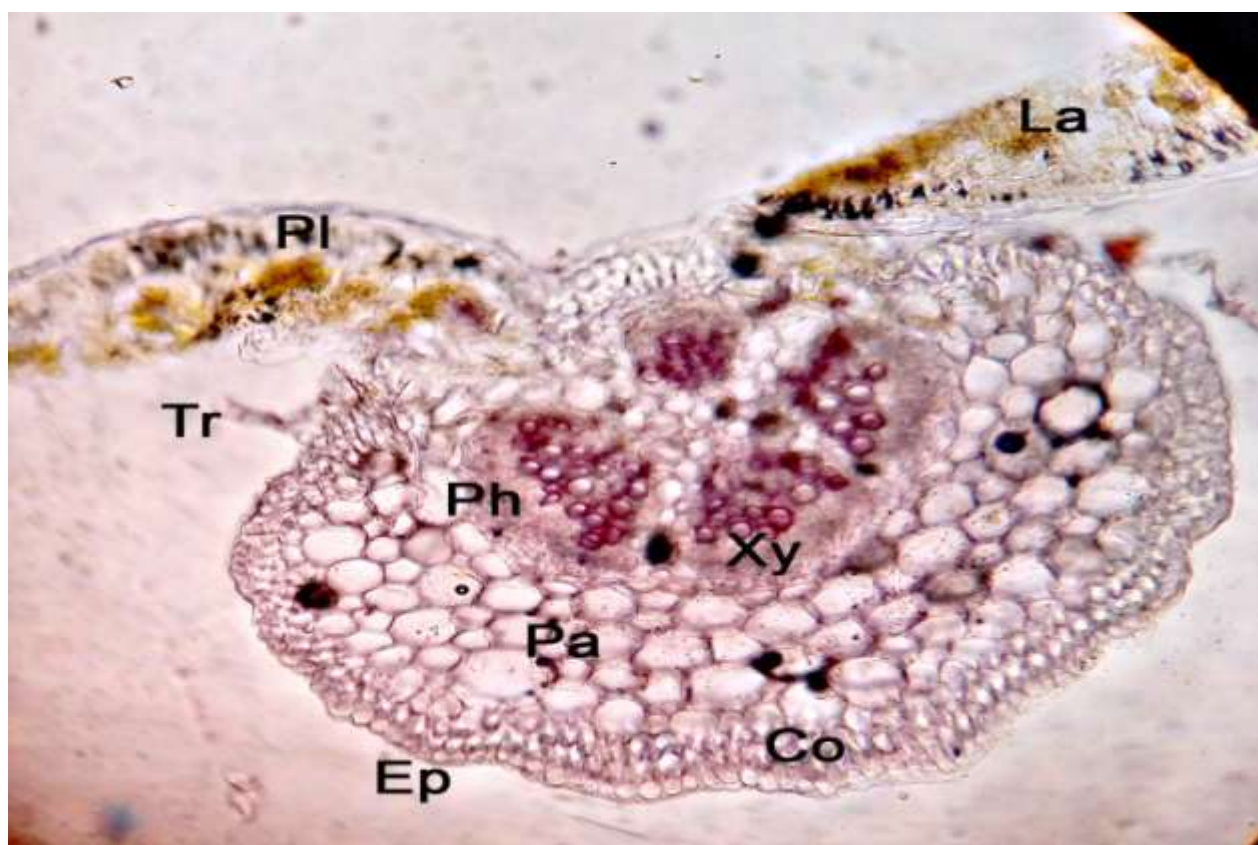
T.S of midrib leaves of *Amaranthus viridis*.L

Fig- 10: T.S of midrib of leaves of *Amaranthus viridis*.L

(Tr: Trichome, Ep: Epidermis, Pa: Parenchyma, Co: Collenchyma, Xy: Xylem, Ph: Phloem,
La: Lamina, Pl: Palisade)

The T.S of midrib shows the following characters:

Epidermis:

It is a single layer of quadrangular parenchymal cell with wavy anticlinal cell walls, covered by cuticle externally. The stomatal pores are seen occasionally. The trichomes are both covering and glandular type.

Collenchyma:

Two to three rows of thick walled, cellulosic collenchymas cells are present both upper and lower epidermis giving mechanical strength to the inner oragans.

Parenchyma:

Polygonal, thick walled, different sized parenchymal cells are seen in the cortical layer often filled with tannins and starch.

Vascular bundle:

Vascular bundle is located exactly in the middle. Made up of four round vascular bundle consist of lignified xylem, non lignified phloem surrounded by lignified sclerenchymatous fibres.

T.S of lamina of the leaves of *Amaranthus viridis*.L



Fig -11: T.S of the lamina of the leaf of *Amaranthus viridis*.L

(Tr: Trichome, Ep: Epidermis, Pa: Palisade Parenchyma, Xy: Xylem, Ph: Phloem, Me: Mesophyll)

Type of the leaf:

Isobilateral. Both upper and lower epidermis are consists of same cellular arrangement.

Upper epidermis:

Single layer of quadrangular parenchyma cells, anitclinal walls, cuticularised, showing covering and glandular trichomes and stomatal pores occasionally.

Palisade parenchyma:

Palisade is a single layer of longitudinally elongated, compactly arranged parenchymal cells. Rich in chlorophyll pigments. They are not continuous in the midrib region. They are replaced by the presence of collenchymas cells in midrib region.

Mesophyll:

Mesophyll is spongy in nature. In-between the palisade parenchyma, three to four rows of spongy parenchyma, thin walled, leaving large inter cellular spaces have been in the mesophyll region. The cells are consist cell inclusion as calcium oxalate crystals and starch grains.

Lower palisade:

Both upper and lower palisade layers are same.

Lower epidermis:

same as upper epidermis. The number of stomatal pores are more in lower epidermis.

Trichomes:

Covering trichomes: multicellular, uniseriate without branches, blunt at apex.

Glandular trichomes: unicellular body and unicellular head.

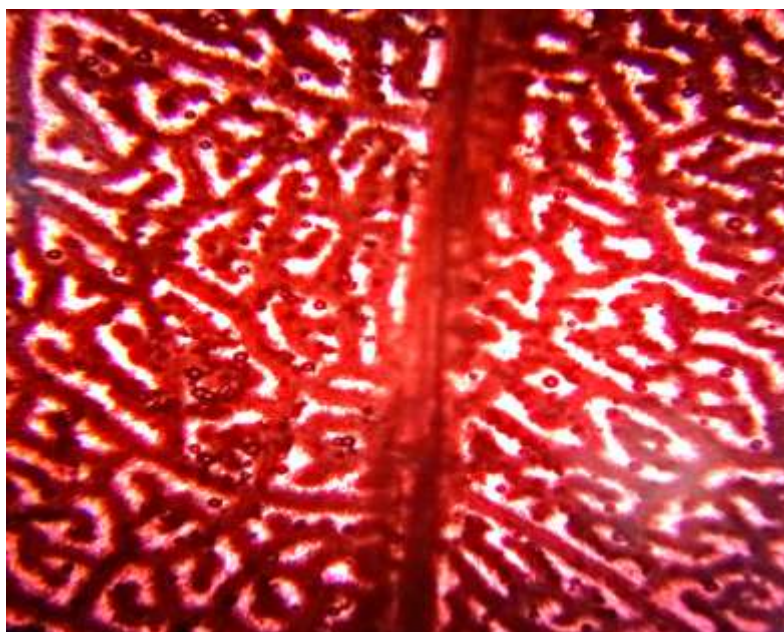
Venation pattern of leaves of *Amaranthus viridis*.L

Fig -12 : venation pattern of leaves of *Amaranthus viridis*.L

Description:

After clearing with clearing reagent, the leaf shows pinnate venation pattern. It shows the present of lateral veins, vein lets, vein islets and vein terminations. It also shows the presence of tannins and calcium salt deposition on the cell wall.

ANALYTICAL PARAMETERS

The analytical parameter were investigated and reported as total ash value (06.3% w/w), Water soluble ash value (1.20% w/w), Acid insoluble ash value (2.60%), Sulphated ash value (2.90% w/w), Water soluble extractive value (12.75% w/w), Alcohol soluble extractive value (12.60% w/w), Loss on drying (8.90% w/w). The above studies were enabled to identify the plant material for future investigation and form an important aspect of drug studies.

Table- 5: The results were in the following table: Data for ash values for powdered leaves of *Amaranthus viridis*.L

S.No	PHYSICAL PARAMETERS (ASH VALUES)	PERCENTAGE (W/W)
1.	Total ash value	06.8W%
2.	Water soluble ash	1.20%
3.	Acid soluble ash	2.60%
4.	Sulphated ash	2.90%

EXTRACTIVE VALUE AND LOSS OF DRYING

Table- 6: Data for extractive value and loss on drying of powdered leaf of *Amaranthus viridis* Linn

S.NO	ANALYTICAL PARAMETERS	PERCENTAGE (W/W)
1.	Alcohol soluble Extractive Value	12.6%
2.	Water soluble Extractive Value	12.75%
3.	Moisture content	8.90%

Table- 7: Results of the phytochemical constituents of Methanolic Extract of *Amaranthus viridis*.L.

S.No	Constituents	Methanolic extract of Leaves of <i>Amaranthus viridis</i> .L.
1.	CARBOHYDRATES	+ Ve
2.	FIXED OILS AND FATS	-Ve
3.	PROTEIN AND AMINO ACID	+ Ve
4.	SAPONINS	+ Ve
5.	STEROIDS	+Ve
6.	ALKALOIDS	+Ve
7.	GLYCOSIDES	+Ve
8.	FLAVONOIDS	+Ve
9.	TANNINS	+Ve
10.	GUM & MUCILAGE	-Ve
11.	TRITERPENOIDS	+Ve
12.	PHENOLIC COMPOUNDS	+Ve

(+) Presence (-) Absence

PHARMACOLOGICAL STUDIES

EVALUATION OF NEUROPROTECTIVE EFFECT:

ANTI-CONVULSANT ACTIVITY

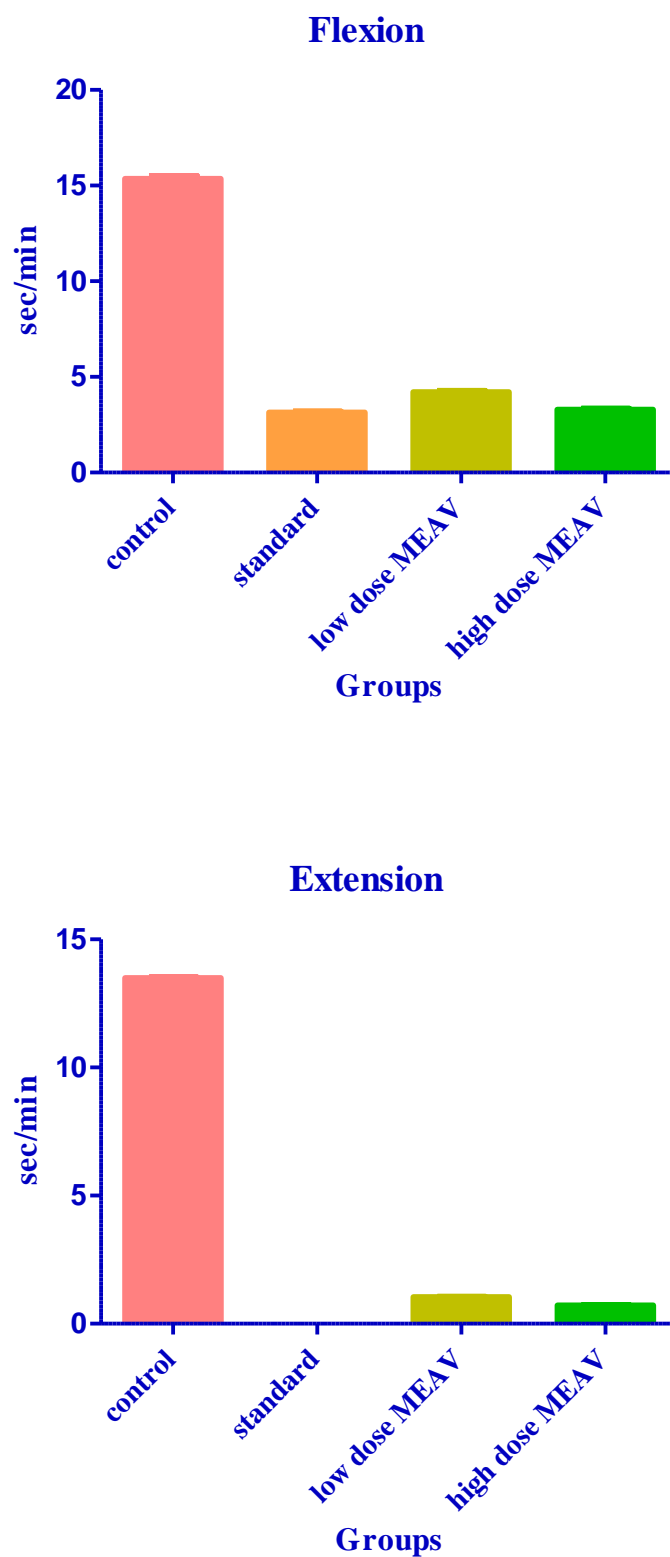
Table- 8: Effect of Methanolic extracts of *Amaranthus viridis*.L. on Maximal electroshock induced in rats

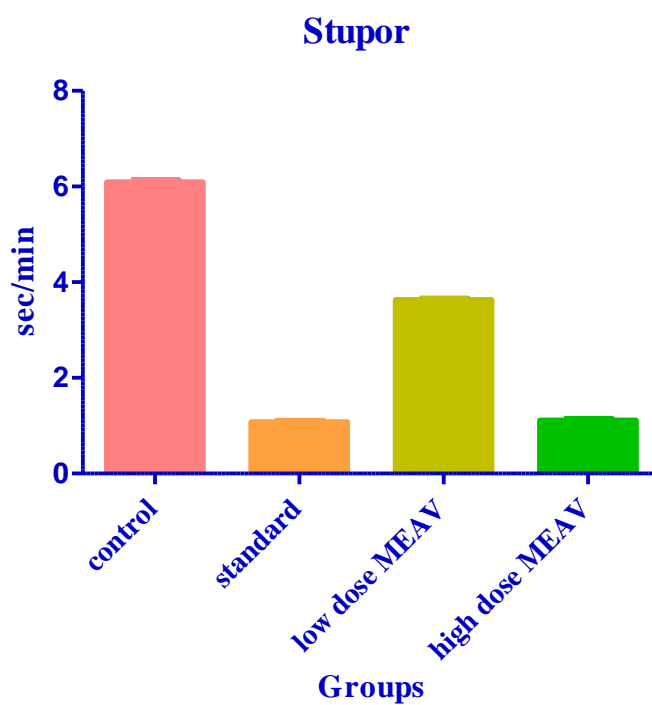
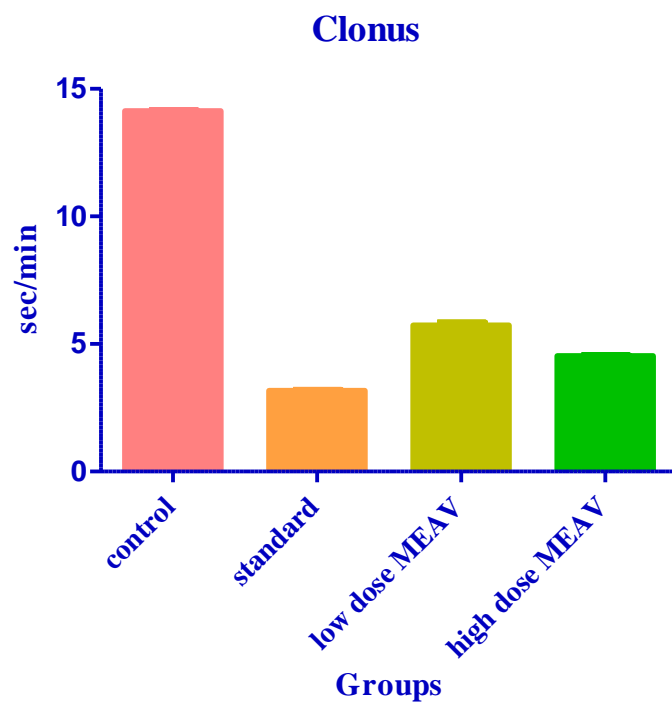
Group	Dose (mg/kg)	Flexion	Extension	Clonus	Stupor	Recovery / Death
Control	Normal saline	15.35±0.157	13.5±0.025	14.13±0.035	6.09±0.039	Recovery
Phenytoin	20 mg/kg	3.14±0.049***	0	3.17±0.036***	1.07±0.023***	Recovery
MEAV	250 mg/kg	4.20±0.067***	1.05±0.010***	5.73±0.108***	3.63±0.025***	Recovery
MEAV	500 mg/kg	3.29±0.049***	0.72±0.014***	4.53±0.028***	1.10±0.026***	Recovery

Note: Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; ns= non-significant; N = 5.

Fig- 8: Diagrammatic representation of Effects of Methanolic Extract of *A. viridis* on Maximal electroshock induce in rats





ANTI-DEPRESSANT ACTIVITY

Forced Swimming Test (TST):

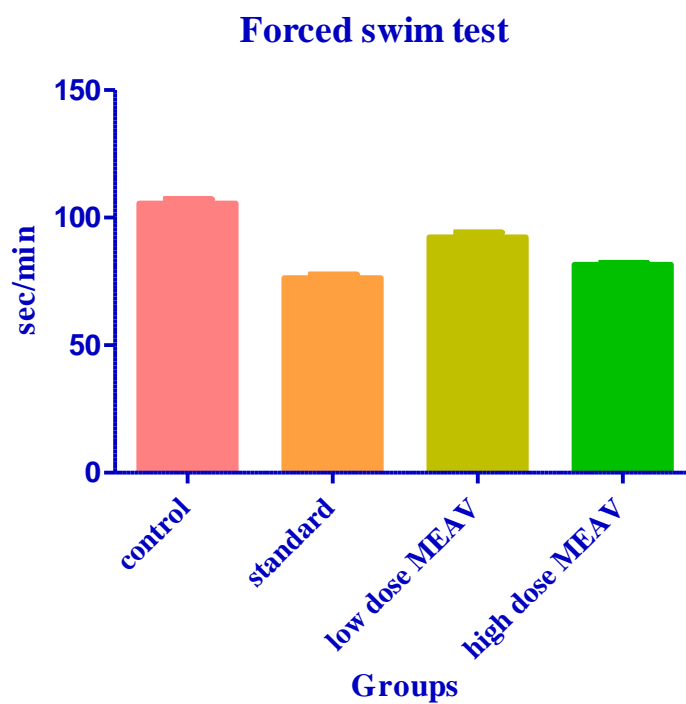
Table- 9: Effect of Methanolic Extracts of *Amaranthus viridis*.L. on forced swimming test in mice

Group	Dose (mg/kg)	Duration of immobility time (sec)
Control	Normal saline	105.5±1.658
Imipramine	60 mg/kg	76.25±1.377***
MEAV	250 mg/kg	92.25±1.887***
MEAV	500 mg/kg	81.5±0.866***

Note: Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; ns= non-significant; N = 5.

Fig: 9- Diagrammatic representation of Effects of Methanolic Extracts of *Amaranthus viridis*.L. on forced swimming test in mice



Tail Suspension Test (TST)**Table- 10: Effect of Methanolic Extracts of *Amaranthus viridis*.L. on Tail suspension test in mice**

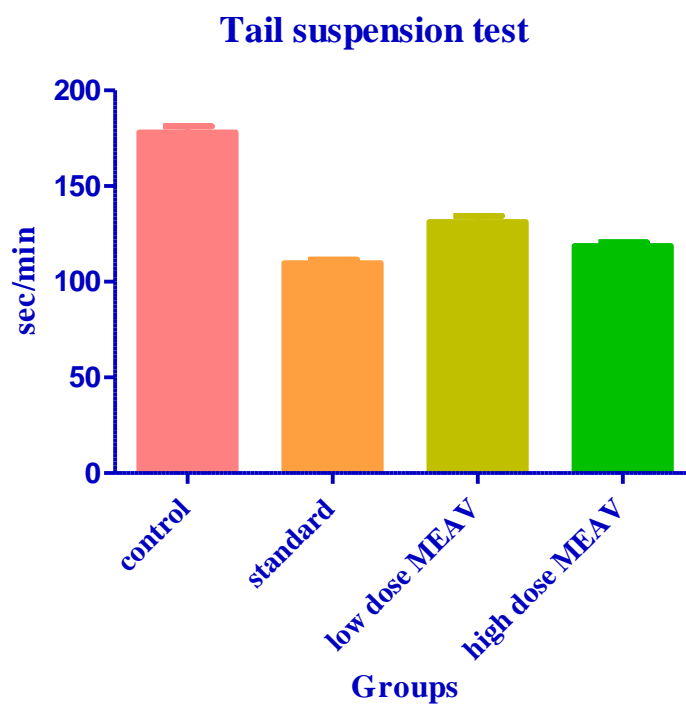
Group	Dose (mg/kg)	Duration of immobility time (sec)
Control	Normal saline	178±3.24
Imipramine	60 mg/kg	109.8±1.75***
MEAV	250 mg/kg	131.3±3.119***
MEAV	500 mg/kg	118.8±1.887***

Note: Values were expressed as mean ± SEM. Statistical analysis was done by one way

ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; ns= non-significant; N = 5.

Fig- 10: Diagrammatic representation of Effects of Methanolic Extracts of *Amaranthus viridis*.L. on Tail suspension test in mice



ANTI-ANXIETY ACTIVITY

Elevated Plus Maze Test

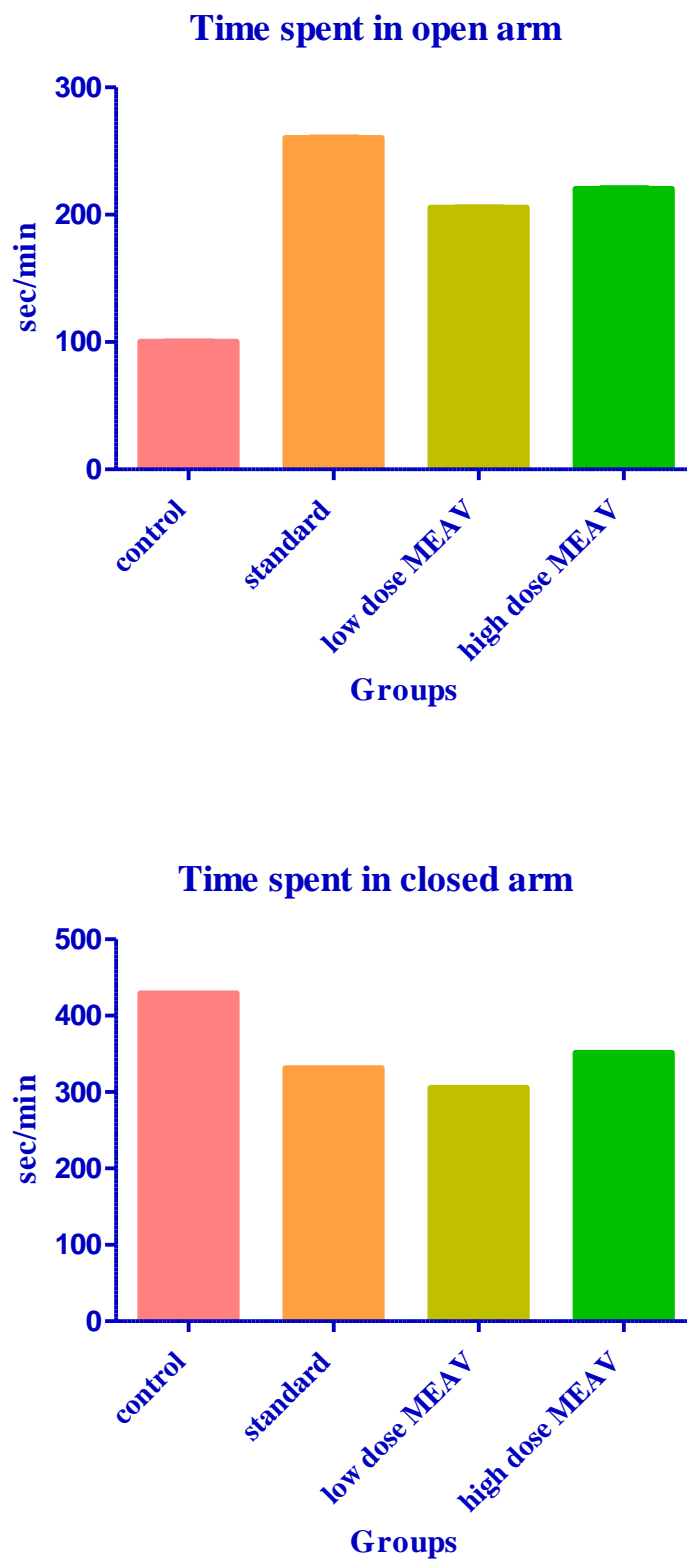
Table -11: Effect of Methanolic Extract of *Amaranthus viridis*.L. on Elevated plus maze (EPM) test in mice

Group	Dose (mg/kg)	Time spent in closed arm (sec)	Time spent in open arm (sec)
Control	Normal saline	429.4±0.1586	100.4±0.1304
Diazepam	4 mg/kg	331.4±0.0312 ns	260.4±0.1493***
MEAV	250 mg/kg	305.5±0.04131 ns	205.5±0.1602**
MEAV	500 mg/kg	351.4±0.1035 ns	220.4±0.2121***

Note: Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; ns= non-significant; N = 5.

Fig- 11: Diagrammatic representation of Effects of Methanolic Extracts of *Amaranthus viridis*.L. on Elevated plus maze (EPM) test in mice



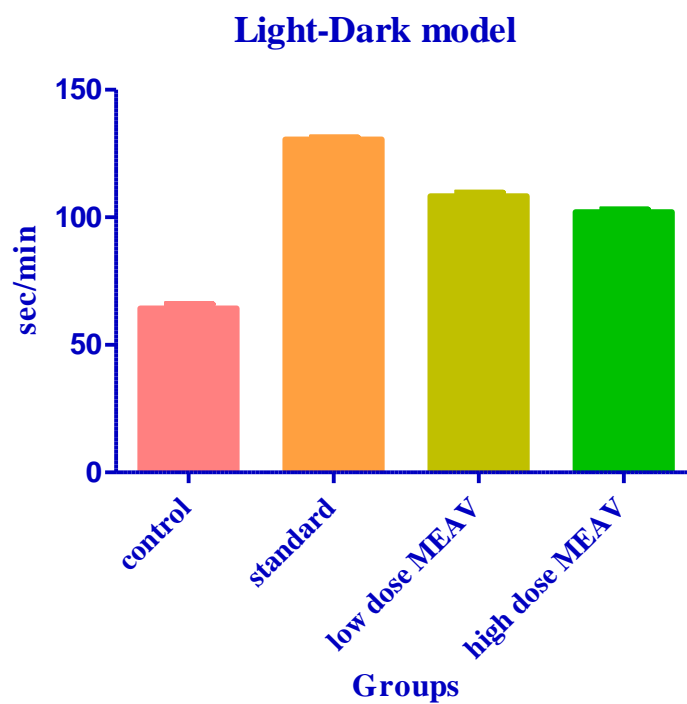
Light- Dark Model:**Table-12: Effect of Methanolic Extract of *Amaranthus viridis*.L. on Light Dark Model in mice**

Group	Dose (mg/kg)	Time spent in the light chamber (sec)
Control	Normal saline	64.25±1.702
Imipramine	60 mg/kg	130.5±0.866***
MEAV	250 mg/kg	108.3±1.436***
MEAV	500 mg/kg	102±1.08***

Note: Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; ns= non-significant; N = 5.

Fig-12: Diagrammatic representation of Effects of *Amaranthus viridis*.L. Methanolic Extracts of *Amaranthus viridis*.L. on Light – Dark model in mice



DISCUSSION

The methanolic extract of leaves of *Amaranthus viridis* linn was evaluated for its neuroprotective activity. Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, glycosides, protein and amino acids, phenolic compounds. The present investigation of the methanolic extract of leaf of *Amaranthus viridis*.L produced central inhibitory effect in mice and rats. Behavioural pharmacology field makes the concepts that are derived from pharmacology and psychology to study behaviour in animal models. The discovery of new compounds which act on CNS process (either CNS depressant or CNS stimulant) will-provide clinical useful information for validation of animals. This will also new insight to researcher to understand the physio-pathological and neurochemical process involved in investigation of new compounds. ^[79]

Advance in modern science and technology have contributed to a massive advancement in the quality of human life. However, contemporary life stresses are accountable for the surge in frequency of diversity of neurologic and psychiatric disorders like anxiety, depression, psychosis, schizophrenia, Parkinsonism, Alzheimer's disease, epilepsy etc. The drugs which are currently used for the treatment of these disorders provide symptomatic relief rather than altering the course of the disease. The Adverse effect of the drugs ranges from exasperating to hindering or potentially incurable effects. Furthermore, the overall functions and eminence of life outcome of patients still remains pitiable after treatment. Thus, for the treatment of diseases, there is a critical need to search for less toxic and more effective drugs. In this context, an increasing number of herbal products have been introduced as an alternative for treating these neurologic and psychiatric disorders. Ayurveda in contrast to the modern western medicine considers detoxification as the prime part of treatment and believes in the fact that ailments continues cracking up again and again as far as the disease causing factors are accessible in the body. The herbal products owing to their

less toxicity and adverse effects when compared with allopathic treatment have gained widespread utility for treatment of neuropsychopharmacological diseases^{[80][81]}

Thus plant extract and herbal medicines have stood the test of time for their wellbeing, effectiveness, adequacy and lesser side effects. The current study deals with the inquiry of the effects of *Amaranathus viridis*, and plant extract in different animal models in the area of neuropsychopharmacology.

Currently available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the patients, another 25% may show improvement where as the remainder does not benefit significantly. Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult so that a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes.

In most common forms of epileptic seizures, effective drugs appear to work either by promoting the inactivated state of voltage activated Na⁺ channels or enhance GABA mediated synaptic inhibition.

The observation of present study indicates that methanolic extract of *Amaranthus viridis*.L (MEAV) possesses anticonvulsant activity in rat. GABA is the major inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy.^[82]

The present study shows that the methanolic extract of leaf of *Amaranthus viridis*.L protected some of the animals against seizures induced by maximal electroshock. Antiepileptic drugs which inhibit voltage-dependent Na⁺ channels, such as phenytoin can prevent MES-induced tonic extension.

In MES-induced convulsion animals represents grandmal type of epilepsy. It has often been stated that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Moreover, MES-induced tonic extension phase can be prevented either by drugs that inhibit voltage-dependent Na⁺ channels, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutaminergic excitation mediated by the N-methyl- D-Aspartate (NMDA) receptor such as felbamate. The MEAV showed anticonvulsant activity against MES-induced convulsion, it was abolished tonic extension phase due to it might be either inhibit voltage-dependent Na⁺ channels or act as a NMDA antagonists.^{[83][84]}

In the result of present study, MEAV (250-500 mg/kg) produced significant antidepressant effect in FST & TST. These models of depression are widely used to screen new antidepressant drugs. The tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including TCAs, SSRIs, MAOI, Atypical antidepressants. The forced swimming test is the most widely used tool for assessing antidepressant activity pre- clinically. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents. It has been argued that TST (Tail Suspension Test) is less stressful than FST (Forced swim test) and has greater pharmacological sensitivity. The results obtained from TST are in concordance with the validated FST by Porsolt et al. Environmental factors and hereditary factors play a major role in producing deficient monoaminergic transmission in central nervous system thereby producing symptoms of depression.^[85]

The EPM is one of the most widely validated tests and is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma amino-butyric acid type A (GABA_A) - benzodiazepine complex. In EPM, normal mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an

aversion towards open arms that is generated by the fears of the open spaces. Drug like diazepam that increases open arm exploration are considered as anxiolytic and the reverse holds true for anxiogenics. In this study, we observed that the administration of different doses (250 and 500 mg/ kg body weight) of methanolic extract of *Amaranthus viridis*.L. induced an anxiolytic-like effect in mice, as it increased open arm entries and the time spent in the open arms of the EPM when compared to the control animals.^[86]

The light –dark test were used to access the anxiolytics potentials of *Amaranthus viridis*.L. In the light-dark test (250-500mh/kg) dose showed the most prominent anxiolytic – like properties by spending the highest time in the light chamber. This corroborate the suggestions that the time mice spent in the illuminated side of the light dark chamber is the most useful and consistent parameter of anxiety.

From the present study, it can be concluded that *Amaranthus viridis*.L. (MEAV) shows significant psychotherapeutics effects as antidepressants and anxiolytics agent. This research work has eliminated the involvement of neurotoxicity in the use of *Amaranthus viridis*.L for pharmacotherapy in anxiety and depression. Detailed laboratory analysis is required for a definitive conclusion and isolation of major active secondary metabolites responsible for these therapeutic actions. So, a potent antidepressants and anxiolytics may emerge from *Amaranthus viridis*. Since the extract shows potent anxiolytic and depressant effects nearly at same dose range hence it could also be used in treatment of mixed anxiety and depression syndrome.^[87]

Results of the present investigation suggest that the extract of *Amaranthus viridis*.L. possesses neuropharmacological activity and provide the scientific basis for the use of the plant in traditional system of medicine in the treatment of nervous disorders.

9. SUMMARY AND CONCLUSION

The present study was undertaken to determine the effect of neuropharmacological effects of leaves of methanolic extract of *Amaranthus viridis*.L

The pharmacognostical studies made on the *Amaranthus viridis*.L determines the various parameters for pharmacognostical standards. The present investigation deals with the report on microscopically, transverse section of *A.viridis* leaves of (petiole, midrib, lamina, venation pattern) and different chemical parameters have been determined. These findings will be towards establishing pharmacognostic standards on identification, purity, quality, classification of the plant, which is gaining relevance in plant drug research. The powdered leaves like ash values, extractive values and loss of drying gave valuable information. This helped for correct identification of plant

The preliminary phytochemical investigation showed the presence of carbohydrates, alkaloids, steroids and sterols, glycosides, saponins, tannins, flavonoids and phenolic compounds

Our conclusion, investigation suggests that methanolic extract of powdered leaves of *Amaranthus viridis*.L. Which possess potent neuroprotective effects in rats & mice.

10. FUTURE PROSPECTIVES

The current study reveals that methanolic extract of *Amaranthus viridis*.L is having neuroprotective activity. So in future we can go for,

1. A lead molecule having the neuroprotective activity can be isolated from the extract of plant *Amaranthus viridis*.L.
2. *A.viridis*.L. is an interesting weed vegetable with a good nutritional value.
3. It certainly deserves more attention to determine wider domestication possibilities and optimum cultivation practices.
4. Its medicinal properties need further investigation as well.
5. More research is needed into the nutritional aspects and utilization of the *Amaranthus viridis*.L.

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